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# *KYNURENINE AS AN INTERMEDIATE IN THE FORMATION OF NICOTINIC ACID FROM TRYPTOPHANE BY NEUROSPORA**

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For a number of years it has been clear that nicotinic acid is synthesized by at least some mammals. The amount of this vitamin retained in the body of a rat plus that excreted may far exceed the dietary intake.[1-3] On a diet containing adequate protein the dog appears not to need exogenous nicotinic acid.[4] Recently it has been shown that nicotinic acid synthesis in the rat,[3, 5-7] man,[8, 9] the horse[10] and the cotton rat[10] is increased when the diet is high in tryptophane.

It has likewise been shown by Woolley[11] and by Kodicek, Carpenter and Harris[12] that nicotinic acid and tryptophane are interchangeable in counteracting the pellagragenic effects of 3-acetyl pyridine, indole-3-acetic acid, and a factor present in corn that may be related to one of these.

As pointed out by Rosen, Huff and Perlzweig[5] these facts suggest that tryptophane may serve as natural precursor of nicotinic acid. Other interpretations are possible, however.[13]

Evidence in support of the hypothesis that tryptophane is a precursor of nicotinic acid and that kynurenine is an intermediate in the conversion comes from a study of a mutant strain of *Neurospora crassa*. This mutant strain, designated 65001, descended from a conidium irradiated with 9400 ergs of ultraviolet per square millimeter. The manner of treatment and of isolating and detecting mutant stains has been described elsewhere.[14]

Mutant strains of *Neurospora* requiring exogenous tryptophane for growth are known.[15] Other strains have been studied that will not grow unless nicotinic acid or a related compound is supplied in the medium.[16] In certain of these strains the requirements are specific. Tryptophaneless strain 10575 will not respond to nicotinic acid, and nicotinic acid-requiring strains 3416 and 4540 will not grow on tryptophane. Strain 65001, on the other hand, grows if supplied with *either* tryptophane or nicotinic acid.

In outcrosses to wild-type strains, mutant 65001 behaves as though it differed from normal by a single gene. Asci from the cross carry four ascospores that give rise to cultures that grow in the absence of both tryptophane and nicotinic acid and four spores that give cultures which, like the mutant parent, require either but not both of these growth factors. The gene in which 65001 and wild type differ is not sex linked. Its genetic relation to genes carried in mutant form by previously reported tryptophaneless and nicotinicless strains has not yet been completely established.

The behavior of strain 65001 suggested to us that it, like the rat, might be able to make nicotinic acid from tryptophane. Kynurenine, known as a product of tryptophane metabolism in mammals,[17,18] insects[19,20] and bacteria,[20] suggested itself as a possible intermediate in this process. Tests of *l*-kynurenine, isolated from the urine of rabbits and kindly made available to us by Professor S. Lepkovsky of the University of California, showed it to be active in promoting growth of mutant 65001. As determined by three-day growth in 125-ml. flask cultures,[16] approximately 1.6 micromoles of *l*-kynurenine gives half-maximum growth. The corresponding values for nicotinamide and *l*-tryptophane are 1.4 and 9.8 micromoles, respectively.

Following the method of Butenandt, *et al.*[21,22] *dl*-kynurenine was synthesized. It proved to have half the activity of the natural material showing that the *d*-isomer is not utilized as a growth factor by 65001.

Like the mammal, strain 65001 produces an excess of nicotinic acid (or amide) when grown in the presence of an excess of tryptophane but not when grown on an amount just sufficient for growth. Thus four 500-ml. flask cultures of 65001, made up with 100 ml. of culture medium each, two containing 2 mg. *dl*-tryptophane per 100 ml. medium and two containing four times as much, were grown for seven days and the culture medium tested for nicotinic acid. The lesser amount of tryptophane was adequate for growth of strain 65001. Following replenishment of sugar and biotin, the culture media were tested for ability to support growth of mutant 3416, a strain which appears to be specific for nicotinic acid or nicotinamide.[16] The media initially high in tryptophane gave good growth while those initially low in tryptophane gave very little. While it is conceivable that the compound accumulated is not nicotinic acid or nicotinamide but a compound of unknown nature with similar biological activity, this seems unlikely.

An experiment made in a similar manner shows that when 65001 is supplied *dl*-kynurenine in an amount just sufficient for maximum growth (500 micrograms per 20-ml. medium), no nicotinic acid is given off in the culture medium in 12 days. But when the kynurenine is increased fourfold, nicotinic acid accumulates in the culture medium in an amount sufficient for maximum growth of strain 3416—at least 0.5 microgram per milliliter of culture medium.

These experiments lend strong support to the view that *Neurospora* converts tryptophane to nicotinic acid by way of kynurenine.

Since kynurenine is active as a growth factor for strain 39401 which was used as an assay strain by Bonner and Beadle[16] in the isolation of two nicotinic acid precursors accumulated by strain 4540, the question arises as to the relation of kynurenine to these precursors. Elementary analyses of the strain 4540 precursors reported by Bonner and Beadle show that they cannot be kynurenine, a conclusion consistent with independent evidence indicating that they both contain a pyridine ring.[16] A reëxamination of the properties of mutant 39401 shows that it, like mutant 65001, grows when supplied tryptophane. These two strains may be alike genetically; attempts to cross them have so far been unsuccessful.

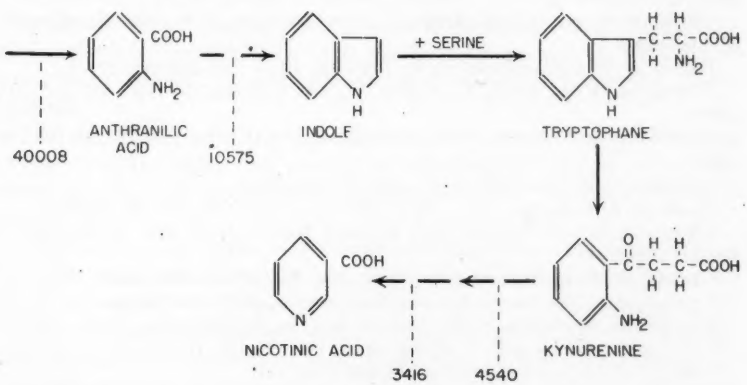


FIGURE 1

Proposed scheme of synthesis of tryptophane and nicotinic acid in Neurospora.

On the basis of the evidence so far available, we suggest that the synthesis of tryptophane and nicotinic acid in *Neurospora* takes place according to the scheme shown in figure 1. It is clear that this scheme is incomplete. The part played in it by the gene in which strain 65001 differs from wild type is not yet entirely clear but we suspect that it is concerned with one of the steps between anthranilic acid and indole. In addition the evidence strongly suggests that one of the functions of nicotinic acid is to act catalytically in the synthesis of tryptophane.

*Summary.*—On the basis of the growth responses of *Neurospora* mutant strains to tryptophane, nicotinic acid and kynurenine, it is postulated that nicotinic acid is normally formed from tryptophane with kynurenine as an intermediate. If this conclusion is correct, kynurenine has a biological significance not heretofore suspected. Whether kynurenine serves as an in-

termediate between tryptophane and nicotinic acid in the mammal remains to be determined.

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## *ON THE EVOLUTION OF THE GENUS NICOTIANA**

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*Taxonomic Status.*—The relatively advanced family *Solanaceae* is allied to such families as the *Labiatae*, *Hydrophyllaceae*, *Boraginaceae* and *Polemoniaceae* but most closely related to the *Nolanaceae* and *Scrophulariaceae*. The approximately seventy-five genera have been organized in five to nine

tribes or subtribes. Don, Endlicher, Miers and Dunal placed *Nicotiana* in the tribe *Nicotianae*, Bentham and Hooker and von Wettstein, in the *Cestr(in)eae*.

In taxonomic organizations of the *Solanaceae* the genera most frequently associated with *Nicotiana* are *Petunia, Cestrum, Sessia, Vestia, Metternichea, Retzia, Fabiana* and *Nierembergia*. From my point of view the morphological evidence more intimately relates the following genera to *Nicotiana: Petunia, Cestrum, Vestia* and *Fabiana*.

A recent taxonomic reorganization of the genus (Goodspeed[1]) adopts the treatment of Don and recognizes three subgenera and eleven sections with a total of fifty-eight species involved. In subgenus *Rustica* there are sections *Paniculatae* (seven species), *Thyrsiflorae* (one species) and *Rusticae* (one species); in subgenus *Tabacum*, sections *Tomentosae* (five species) and *Genuinae* (one species); in subgenus *Petunioides*, sections *Undulatae* (three species), *Trigonophyllae* (two species), *Alatae* (nine species), *Noctiflorae* (four species), *Acuminatae* (ten species) and *Suaveolentes* (fifteen species).

The significance with regard to evolutionary mechanisms in *Nicotiana* of certain of the inter- and intrageneric relationships defined in the taxonomic status of the genus briefly summarized above will appear in what follows.

*Morphology.—Nicotiana* is a conservative genus in the sense that it does not exhibit characteristic morphological specialization of particular floral or vegetative organs. On the other hand, no other genus of the family, save possibly *Solanum*, shows a greater range of variation in habit, inflorescence and flower than does *Nicotiana*, and the degree of physiological specialization attained by many of its species indicates the extent to which they have evolved.

Between the extremes represented, on the one hand, by desert ephemerals a few inches high and, on the other, by shrubby to subarborescent xerophytic or subtropical perennials to twenty-five feet in height are robust annuals some of which become limited perennials in favorable environments, and also root perennials spontaneously propagating from underground parts regardless of the fate of aerial ones. Inflorescence types include a thyrse panicle and a flat spray, between which complex extremes lie such intermediates and specializations as mono-, di- and pleiochasial cymes, solitary flowers and inconstant, diffuse paniculate-cymose mixtures.

Variability of the flower is chiefly expressed in the form and color of the corolla, stamen insertion and aestivation. In some species the flower is vespertine. The corolla is essentially salverform to trumpetform or tubular, the limb entire to rather deeply lobed. Red, green and yellow in various intensities and combinations occur. Pure white is found in only one species while a corolla predominantly white with a green to purplish flush

exteriorly is common. The stamens may be included or exserted, equal or unequal, long or short and inserted at any point from near the base of the corolla to immediately below the limb.

Consideration of major and minor details of habit, inflorescence and flower of *Nicotiana* species reveals correlations suggesting five definite but interrelated morphological nuclei or clusters of species. The *rusticoid* and *tomentosoid* nuclei can be related by shrubby habit, thyrse inflorescence, non-vespertine corolla with tendency for the limb to pass slowly through an upright, horizontal and recurved condition, and low insertion of stamens. The other three—the *alatoid*, *noctifloroid* and *corymbosoid*—also show interrelation. They are herbaceous nuclei; the inflorescence is variable but not a thyrse; the flower is usually whitish, vespertine and salverform; the limb seldom passes through a series of orientations; stamen insertion is higher, sometimes apical.

The *rusticoid* nucleus (subgenus *Rustica* in general, and a group of its member species in particular) shows greatest regularity in leaf form, size and distribution; evenness in distribution of branches; an inflorescence derivable from a leafy shoot and capable of producing other known inflorescence types in the genus by reduction; pronounced regularity of all four whorls of the flower; relatively little adnation of filaments; a conservative condition of corolla throat, stamens, pistil and hypogynous disc. On morphological grounds it is, therefore, taken to represent that portion of the genus in which the most primitive characters have been preserved. By contrast with various species of the *tomentosoid* and *corymbosoid* nuclei, the more strictly *rusticoid* members of subgenus *Rustica* exhibit relatively little polymorphy; the same is true of most members of the *alatoid* nucleus (including species of section *Alatae*) and less true of the *noctifloroid* (including species of section *Noctiflorae*).

The morphological evidence points to the rôle of hybridization in the origin of the five modern nuclei. Thus, one may assume that on a primitive ancestral level a series of progenitors of genera including pre*Nicotiana*, pre*Petunia*, pre*Cestrum* and the ultimate ancestors of other genera now related to *Nicotiana* began to vary each about a distinct morphological mean—differentiation referable to genic and structural alterations accompanied by combination and recombination, selection and genetic and geographic isolation. Two ancestral complexes, the *glaucoid* and the *petunioid*, appeared as a result of hybridization of pre*Nicotiana* with, in the first case, pre*Cestrum* and, in the second, pre*Petunia*, followed by differentiation within two diverging modes of morphological variation thus established. Most of the members of the modern *rusticoid* and *tomentosoid* nuclei arose from the *glaucoid* complex, while, to the same degree, the *alatoid*, *noctifloroid* and *corymbosoid* nuclei were derived from the *petunioid* complex. To this point and to this extent a dendroid diagram of the evolution of

*Nicotiana* might be constructed. However, certain species and species groups within and without the five nuclei cut sharply across such a diagram and transform it into a reticulum. Thus, there are modern species which owe their origin to hybridization between derivatives of the *glaucoid* and *petunioid* complexes, and others, to hybridization between components of those ancestral complexes themselves.

*Distribution.*—Today the distribution of the genus is essentially tripartite: South America, North America, Australia and the South Pacific. In South America *Nicotiana* occurs in approximately the entire "tail" of the continent, and also in southern and western Peru, the drier southwest of Ecuador, parts of Bolivia and the southern portion of western Brazil, but is absent from the drainage systems of the Amazon and Oronoco and from the northern countries of the continental "shoulder." In North America it is found native in central and northern Mexico and in the western United States. Scattered stations are known in Guatemala and the West Indies. In Australia it has been collected in all but the tropical north and northwest. In the South Pacific it extends in isolated localities from New Caledonia to the mid-Pacific Marquesas. This distributional picture is a product of the fact that, in general, modern species of *Nicotiana* demand light, a well-drained soil and dry rather than humid heat. Only a few species have set aside one or another of these requirements and occur on the margin of the moist tropics.

Presumably in South America existed the original reservoir from which the present-day assemblage of *Nicotiana* species arose. Certainly there is striking numerical preponderance of South American species and the extent of their differentiation into morphologically and cytogenetically distinct subdivisions has no parallel in the other two distributional areas. More specifically the center of distribution is taken to be the region now represented by the general area of junction of Peru, Bolivia, Argentina and Chile which corresponds to the current distribution of the *rusticoid* nucleus shown above to represent, on morphological grounds, the most primitive of the five clusters of modern species.

The tripartite, geographically discontinuous distribution of the genus is explicable on the assumption that ecologically suitable paths for migration connected South America with each of the other two continents in which species of *Nicotiana* are today native. The obvious suggestion that such a pathway to Australia included Antarctica and Tasmania is supported by the tracing of an extension of the Andean system into the Antarctic continent, the indication that a temperate flora existed there at a relatively recent time and that migration from Antarctica to Australia via Tasmania occurred. The problem of a route permitting passage from South to North America involves the semixerophytic nature of most modern *Nicotiana* species and the tropical zone which now separates the two

continents. There may have been a Caribbean path followed by certain progenitors more tolerant than current species of semitropical environments. Along it they could have passed from the northern "shoulder" of South America through the arc of the West Indies to Mexico, a route to which the distribution of other plants points more clearly. On the other hand, if the considerable decrease in temperature postulated for Central America during the glacial epoch induced a somewhat dry subtropical climate, migration might largely have followed the path of today's land connection between the two Americas. From central California to Mexico and from Chile to southern Peru a previously greater westerly extension of land has been postulated. Had a similar extension once existed between Mexico and Ecuador another pathway between central western South America and southwestern North America might have become continuous. In these various connections it is to be noted that both the western United States and Australia have undergone considerable desiccation since the presumptive advent of *Nicotiana*. In the former case this has encouraged the northerly spread of the genus; in the latter it has stranded, segregated and specialized forms already present. The South Pacific localities are considered (Wheeler[2]) to represent eastern migration from the Australian coast probably involving in the extreme instance—the Marquesas—introduction by man.

The distributional evidence indicates that the genus *Nicotiana* has maintained itself from at least a period prior to the formation of an effective Andean barrier, was present in Antarctica when that continent supported a temperate, herbaceous angiosperm flora, and passed from South to North America. It has survived land mass alterations of great magnitude which played a rôle in transforming a restricted, continuous area of distribution into a large, tricontinental and discontinuous one, and were responsible for forcing colonization of new areas and adaptation to new environments upon individual species, as well as for the extinction of others.

*Chromosome Number.*—In the *Solanaceae* haploid numbers from 7 to 36 have been reported; in the *Scrophulariaceae* the range is from 6 to 30. In the *Solanaceae* over fifty-five per cent of the species investigated show 12 pairs while a multiple of 12 pairs occurs in an additional twenty per cent. In the *Nolanaceae* all the five species counted have 12 pairs and in the *Scrophulariaceae* species with 12 as well as species with 6 pairs occur.

Of the fifty-eight species of *Nicotiana* the chromosome numbers of fifty-five are known. Twenty-eight species show 12 pairs; eleven, 24; four, 16; three, 9; two, 10; two, 19; two, 20; in one species each the haploid numbers are 18, 21 and 22. In related genera the known haploid numbers are: 7 (*Petunia*), 8 (*Cestrum, Vestia*), 9 (*Petunia, Fabiana, Nierembergia*), 22 (*Salpiglossis*).

Subgenus *Rustica* and subgenus *Tabacum* contain only species with 12 or 24 pairs. In the third subgenus (*Petunioides*) four of the six sections contain species showing 24 pairs and all but one section species showing 12 pairs. Two aneuploid series occur, each in a separate section of subgenus *Petunioides:* 9 to 12 pairs in section *Alatae* and 16 to 24 pairs in section *Suaveolentes*.

The rôle of hybridization in *Nicotiana* phylesis suggested by the morphological evidence offers a partial explanation of the origin and evolution of the present-day chromosome number situation just summarized. The primitive ancestral reservoir above referred to which included at least pre-*Nicotiana*, pre*Cestrum* and pre*Petunia*, and which via hybridization on the pregeneric level gave rise to the basic *Nicotiana* complexes, the *glaucoid* and the *petunioid*, is assumed to have been 6-paired. From the two 6-paired complexes a 12-paired *Nicotiana* level arose by interspecific hybridization followed by chromosome doubling and, to a lesser extent, by autoploidy. During the establishment of the 12-paired level a residue of 6-paired species, now extinct, was also in existence. Finally, from the 12-paired level, an almost exclusively alloploid 24-paired level was developed, thus contributing largely to the transformation of a basically dendroid into a reticulate diagram of evolution of *Nicotiana*.

Two aneuploid sequences arose, one between the 6- and 12-paired levels, the other below the 24-paired level. The former is today represented only by 9- and 10-paired species, all of them in section *Alatae*, although earlier existence of 8-paired species may be reflected, as will be indicated below, in the occurrence of the other (16- to 24-paired) aneuploid series. In this connection it is to be recalled that 7-, 8-, 9- and 22- ($x = 11$) paired species are found in modern genera related to *Nicotiana*.

The postulated lower numbers in the 6- to 12-paired aneuploid sequence presumably had a sesquidiploid origin. The higher numbers were doubtless derived by reduction from the 12-paired condition, perhaps in part involving reciprocal translocation (cf. Babcock[3]). There are a number of explanations of the origin of the 16- to 24-paired aneuploid series, all species of which are members of the section *Suaveolentes*. In my opinion two or more 24-paired ancestors of section *Suaveolentes* arose, as did the nine modern 24-paired species, by amphiploidy on the early 12-paired level. During a corresponding period, the same or related 12-paired species, crossing with certain of the not yet extinct 6-paired species, produced by chromosome doubling the 18-paired condition which by reduction initiated the 16-paired group of species. Such establishment of the 16-, 18- and 24-paired progenitors of section *Suaveolentes* was followed by the production of 19-, 20-, 21- and 22-paired species as a result of hybridization, sesquidiploidy, addition through fragmentation or reduction by loss or fusion. Other suggested explanations (Kostoff[4]) involve now extinct 8-, 9- and 10-

paired species in the amphiploid production of 16-, 18- and 20-paired species or take 16- and 20-paired species to be secondary derivatives from an 18-paired one so derived. Yet another (Wheeler[5]) assumes the former existence of a series of 8-paired species which by successive amphiploidy gave rise to the 16- and then the 24-paired condition, with subsequent hybridization between the products resulting in the 18- to 22-paired sequence. In any case, it appears that this modern aneuploid sequence represents a series of secondary derivatives with amphiploidy the fundamental mechanism responsible for their origin.

*Chromosome Morphology.*—For the genus as a whole a combination of median (*m*) and submedian (*sm*) chromosomes predominates over subterminal (*st*) ones in the ratio of five to three. In each of the three subgenera there is a distinctive ratio of *m* plus *sm* to *st:* in subgenus *Rustica* it is 9:1, in subgenus *Tabacum* 5:2 and in subgenus *Petunioides* 4:3. In addition, there is a basic karyotype characteristic of those species which represent the morphological core of each section. In other words, chromosome morphology in *Nicotiana* exhibits sufficiently distinctive features to have phylogenetic significance (Goodspeed[6]).

In the species of genera related to *Nicotiana* for which chromosome morphology is known, the genoms consist almost exclusively of *m* or *sm* chromosomes. Thus, in species investigated of both *Cestrum* and *Vestia* all of the chromosomes are *m* or approximately *m*, and in 7-paired species of *Petunia* 5 are *m*, 1 is *sm* and 1 is *st*.

The contention that primitive groups tend to possess predominantly *m* chromosomes while those of more advanced status show an increasing proportion of heterobrachial ones is apparently borne out in *Nicotiana*. Based upon this contention, the above postulated aggregation of 6-paired ancestral species probably possessed all *m* chromosomes. Subsequent evolutionary levels, progressive in terms of chromosome multiplication and with aneuploidy as their by-products, were in most instances characterized by differentiation in chromosome morphology, particularly at the 12-paired level. Thus, although the *rusticoid* derivatives (species of subgenus *Rustica*) of the *glaucoid* complex have preserved almost exclusively the primitive *m* type of chromosome, its *tomentosoid* derivatives (species of subgenus *Tabacum*), derivatives of the *petunioid* complex (species of subgenus *Petunioides*) and species derived by hybridization between the two complexes themselves today combine *m*, *sm* and *st* chromosomes in varying proportions. In the *rusticoid* nucleus the tendency toward preservation of the postulated ancestral *m* type of chromosome may be a product of the initial setting apart of a relatively more uniform, and thus more stable, type of internal chromosomal structure, perhaps genically conditioned, together with the persistence of relatively uniform or favorable environmental conditions.

There is evidence suggesting the direction which possible future alteration of the typical *m* karyotype of subgenus *Rustica* may follow. In all 12-paired species of *Nicotiana* at least four nucleoli are seen, and apart from those of subgenus *Rustica* the majority of the species correspondingly show four satellites. In seven of the eight 12-paired species of subgenus *Rustica* only two satellites appear. However, in each of them at least one *sm* chromosome consistently shows a distinct tertiary constriction which is presumably associated with a nucleolus organizer. Such chromosomes, by fragmentation at the distal portion of the less stable heterochromatic regions doubtless associated with these tertiary constrictions, might be expected to become transformed into *st* satellited chromosomes. As a product of an equivalent internal structural differentiation followed by fragmentation the full complement of satellited chromosomes characteristic of the majority of the species of subgenus *Tabacum* and subgenus *Petunioides* could have been derived. The transitional position in terms of satellite evolution which subgenus *Rustica* apparently occupies would argue in favor of its primitive status, one which is indicated on other grounds.

*Meiotic Chromosome Behavior.*—Complete pairing between structurally homologous chromosomes is shown at *MI* in the majority of species. In certain 12-paired species, however, bridges and fragments at *AI*, and in others frequent occurrence of non-conjunction or non-disjunction followed by irregular distribution of chromosomes, give evidence of the structural hybridity of their genoms. In only one species, an amphiploid on the 24-paired level, has the presence of multivalents been observed. Study of meiotic behavior in races of at least one polymorphic species demonstrates that certain of these races differ from one another by one to several reciprocal translocations and suggests the rôle of structural alterations in initiating species differentiation in *Nicotiana*.

All of the twenty-four monosomics of *N. tabacum* (Clausen and Cameron[7]) and all of the twelve primary trisomics of *N. sylvestris* (Goodspeed and Avery[8]), as well as numerous other chromosomal variants involving loss or addition of one to several chromosomes have been studied in respect to meiotic chromosome behavior. These and similar investigation of variants involving reduction or multiplication of entire genoms—haploids, triploids and tetraploids—have yielded evidence of evolutionary significance. For example, the conspicuous morphological distinctions characterizing each of the primary trisomics of a single species demonstrate the extent to which such chromosome unbalance may be reflected in differences in morphological expression. For example, again, the occurrence at *MI* in tetraploid forms of a number of 12-paired species of valencies higher than four (Fardy and Hitier[9]) suggests the compound character of the diploid representatives of those races.

Meiotic chromosome behavior at *MI* has been studied in a total of two

hundred and thirteen $F_1$ interspecific hybrids of *Nicotiana*. While this number of hybrids represents but a small percentage of the possible combinations of the fifty-eight species of the genus, they include representative combinations pertinent for interpretation of relationships: fifty-three species are involved in the eighty-three intrasectional, forty-nine intersectional and eighty-one intersubgeneric hybrids analyzed.

On the basis of the results of these *MI* analyses the two hundred and thirteen $F_1$ interspecific hybrids may be classified into five categories: lack of pairing, complete, low variable, high variable and "Drosera Scheme" pairing (cf. Goodspeed[10]). In the high and low variable categories where an extremely wide range in number of pairs may occur the high point in the range is considered significant as indicating the maximum number of appreciable homologous segments in the two genoms involved, while the mode may be an expression of normal expectancy on the basis of the maximum number of homologous segments present or may indicate the number of chromosome pairs possessing relatively large pairing blocks. Similarly, in the lack of pairing hybrids with a range of zero to 4 (frequently 0 to 1, 2 or 3), mode zero, the occurrence of up to 4 pairs presumably represents the maximum, though not consistent, expression of small homologous segments residual in the genoms involved, while the characteristic mode of zero is largely a reflection of the lack of pairing blocks of appreciable size and effectiveness. The presence of a limited number of non-homologous segments in the genoms of the two parental species may explain the occasional occurrence of univalents in a hybrid in which the mode is complete pairing. Therefore, on the basic assumption of the significance of pairing (cf. Stebbins[11]), the maximum extent of pairing in an $F_1$ interspecific hybrid may be considered as an index of the relationship of the species involved in terms of their residua of ancestral homology. Such application of extent of pairing as a criterion of relationship in *Nicotiana* largely confirms the morphological evidence expressed in the taxonomic organization of the genus. Thus, in approximately ninety per cent of the $F_1$ hybrids investigated amount of pairing parallels extent of taxonomic relationship of the parental species. In the ten per cent in which extent of pairing does not correspond to degree of taxonomic relationship some few show higher, while the majority show lower, than the amount of pairing to be anticipated. Exceptions of the former type furnish an illustration of the value of a pairing check on a taxonomic organization. Although genetic alterations, reflected in morphological distinctions setting apart two species, have occurred, genically they have more in common than other species of a similar degree of morphological differentiation which show negligible pairing in the $F_1$ hybrid between them. This means that, in the one case as contrasted with the other, there has been less fundamental divergence from a common (or related) ancestor. On the other hand, lower pairing in $F_1$ than

corresponds to degree of taxonomic relationship between the two parental species may be attributed to one or more of the factors recognized as contributing to secondary disturbance of conjugation.

In terms of hybrids which reach maturity and thus are available for cytological analysis *Nicotiana* is apparently exceptional, at least among genera extensively investigated, in degree of compatibility between its species. Thus, relative remoteness in relationship is not a bar to compatibility, as indicated, first by the fact that approximately sixty-five per cent of $F_1$ interspecific hybrids show meiotic irregularities, and second, as noted above, that the same proportion involve as parents members of different subgenera or sections. In other words, it appears that factors restricting compatibility have evolved less rapidly than those inhibiting pairing and those responsible for specific morphological distinctions. Certainly in *Nicotiana* high degree of compatibility is characteristie of many present-day species which have diverged considerably in respect both to external morphology and to extent of pairing in $F_1$ hybrids between them.

In some cases both, and in all cases one, of the species related to the 12-paired ancestry of each of the nine modern American species on the 24-paired level can be identified by meiotic chromosome behavior in appropriate $F_1$ interspecific hybrids. For three of the nine an amphiploid origin is recognized, with identification of the present-day 12-paired descendants of both the original parental races provided in large part by the "Drosera Scheme" pairing relations exhibited by the hybrids between these descendants and the 24-paired species in question. Of these three, *N. arentsii* furnishes a demonstration of an evolutionary sequence initiated by amphiploidy which is today in progress. Thus, the descendants—*N. undulata* and *N. wigandioides*—of the two 12-paired races which entered into its amphiploid origin possess in their genoms sufficient homologous segments to produce a considerable number of bivalents at *MI* of the $F_1$ hybrid between them. Therefore, the original parents represented only certain extents of differentiation, both genic and structural, from a common ancestor or closely related ancestors on the 12-paired level. Consistent with this proposition is the occurrence in *N. arentsii* of multivalents which also appear frequently at *MI* in $F_1$ hybrids between that species and *N. undulata* and *N. wigandioides*. By contrast, *N. tabacum*, another 24-paired species of known amphiploid origin, combines in its ancestry progenitors of two 12-paired species—*N. sylvestris* and *N. otophora* or another member of the same section—which are today taxonomically remote. Correspondingly, the $F_1$ hybrid between these two 12-paired species shows almost complete lack of pairing and no multivalents appear at *MI* in *N. tabacum*. This evidence indicates that the considerable degree of multivalency exhibited by $F_1$ hybrids between *N. tabacum* and both *N. sylvestris* and *N. otophora* (or its relatives) is referable to translocations within the genoms

of *N. sylvestris* and of *N. otophora* or within the genom of *N. tabacum* during the evolution of these three species.

For the remaining six 24-paired American species an amphiploid origin is indicated by "Drosera Scheme" pairing or approximation thereof at *MI* in $F_1$ hybrids between each of these species and a specific 12-paired species. In each case the modern descendants of the other original parent on the 12-paired level have presumably become extinct. However, in some instances morphological and in others cytogenetic evidence points to the position in the author's taxonomic organization of the genus which those species would have occupied. Both types of evidence suggest for certain pairs of modern 24-paired species one common 12-paired ancestor, in some instances represented by descendants today, in others not so represented.

The group of related species including *N. repanda*, *N. nesophila* and *N. stocktonii* provides a present-day illustration of species differentiation from a common ancestor on the 24-paired level. Morphologically the distinctions within the group pattern are considerable but complete pairing in $F_1$ intragroup hybrids occurs. The case of *N. arentsii*, discussed above, provides a possible picture of the origin of such species groups since the products and by-products of multivalent formation, including substitution between members of subgenoms, to be anticipated in the evolution of *N. arentsii* could initiate species differentiation.

Processes similar to those known to be responsible for origin of the 24-paired level from progenitors of known 12-paired species were presumably operative in initiating the 12-paired level from postulated 6-paired ancestral species (cf. Kostoff[4]). Parallel origin from a primitive 6-paired ancestral level not only of pre*Nicotiana* but also of, at least, pre*Cestrum* and pre*Petunia* permitted inter-pregeneric hybridization. In part the product of such hybridization on the next level of differentiation, when those various genera were establishing their distinct modes of morphological variation, was, as already noted, the definition of the 6-paired *glaucoid* and *petunioid* complexes in *Nicotiana*. A subsequent period of expansion of these two complexes gave a series of species whose ranges of morphological variation in some instances did and in others did not appreciably overlap. Correspondingly, their genoms became to different extents mutually exclusive in terms of genic content and structural organization.

Crossing between 6-paired races of various degrees of differentiation from the pre*Nicotiana* level onward, followed by chromosome doubling, was doubtless a determining factor in the origin of the 12-paired level, just as such amphiploidy has been largely responsible for the origin of the modern 24-paired level. On this assumption, the extent of fundamental relationship between 6-paired ancestors plus accumulation of genic and structural distinctions since establishment of the 12-paired level are reflected in the amount and character of pairing at *MI* of $F_1$ hybrids be-

tween present-day 12-paired species. Thus, complete pairing in such hybrids may be interpreted as the expression of an originally maximum degree of relationship together with minimum later accumulation of genic and structural alterations. The opposite extreme, almost complete lack of pairing, would then be referable to minimum initial relationship plus maximum incidence of distinctions subsequently produced, while low and high variable pairing indicate intermediate expressions of the two factors.

Obviously, the possibility of an auto- as contrasted with an essentially alloploid origin of certain members of the original 12-paired from the 6-paired level cannot be excluded. The occurrence of multivalency in current 12-paired species or evidence of autosyndesis in $F_1$ hybrids between them should follow such origin. The first of these consequences has not, in my experience, been demonstrated and evidence concerning the second would be difficult to obtain because of lack of large morphological distinctions at *MI* between members of the genoms of *Nicotiana* species. On the other hand, it is possible that autosyndesis, a product of autoploid origin of one or both parents, may explain in certain instances high variable pairing in $F_1$ hybrids between modern 12-paired species. A 6-paired ancestor in common between two such species, each originating by alloploidy, could be reflected in low variable pairing. In any case, pairing variability is doubtless a product largely of differentiation after attainment of the 12-paired level which has served to reduce the size and alter the arrangement of originally extensive homologous segments.

*Conclusion.*—The point of view presented here concerning the evolution of *Nicotiana* emphasizes the rôle which hybridization has played in establishing the modern expression of the genus, assuming the existence of a now extinct reservoir of 6-paired pre*Nicotiana* races from which the progenitors of modern 12-paired and 24-paired species levels were derived, largely by amphiploidy. Differentiation on the 6-paired level produced two basic ancestral complexes in the development of which hybridization between pre-*Nicotiana* and at least pre*Cestrum* and pre*Petunia* was concerned. From these complexes was produced a series of 12-paired derivatives certain of which are united in the subdivisions of the author's taxonomic treatment of the genus while others trace their origin more directly to both the ancestral complexes via hybridization between their components or immediate derivatives. The present-day 24-paired species level arose largely by amphiploidy involving members of most of the 12-paired clusters of species into which the ancestral complexes became segregated. Throughout the evolutionary sequence genic and structural chromosomal alterations played a part at first more, and later less, definitive than amphiploidy in producing differentiation at successive levels, culminating in the range of morphological variation exhibited by the current *Nicotiana* species assemblage. Documentation of these propositions is afforded by the combined taxonomic,

morphological, distributional and cytological evidence, the most pertinent aspects of which have been briefly outlined above.

In evaluating the present and predicting the future evolutionary status of *Nicotiana* it is to be borne in mind that evidence indicating antiquity does not necessarily indicate senescence from the genetic point of view. Although the extent of morphological, and especially physiological, specialization attained by many species of *Nicotiana* suggests a point in the generic cycle past maturity, the degree of polymorphy indicates a plasticity and capacity for expansion characteristic of a relatively aggressive stage of the cycle (Clausen, Keck and Hiesey[12]). The degree of polyploidy already attained by the genus, a product of the continuing and definitive rôle of hybridization in its evolutionary history, might argue for its advanced or even senescent status. On the other hand, it appears that hybridization with the genom or the gene as the unit of differentiation remains a potentially effective mode of future evolution because apparently genetic barriers are accumulating less rapidly than genic alterations determining species distinctions, with the result that there is a high degree of intersubgeneric as well as intersectional and intrasectional crossability. While the present-day range of distribution of the genus in terms of species is, in general, locally restricted and apparently not increasing, the tripartite nature of its global spread argues against the possibility of extinction since, obviously, future climatic or other alterations would be unlikely to be operative over a sufficient geographic area to eliminate all its species. Indeed, on the assumption that the early part of an interglacial period now obtains, the future extension of a genus, most species of which are arid or semiarid types, would be favored by increasingly warmer, drier climates. In other words, despite the fact that numbers of species of *Nicotiana* have doubtless become extinct, that others have become extremely restricted in extent, and that many are today polyploids exhibiting considerable specialization, survival of *Nicotiana* as a genus is favored by its relatively wide range in distribution and morphological character together with its lack of barriers to hybridization.

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## *ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES I. Pleurotus griseus**

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In a survey[1] conducted in this laboratory it was noted that 213 of the 332 species of Basidiomycetes studied produced substances that inhibited the growth of *Staphylococcus aureus*. Among the species that seemed worthy of further investigation was *Pleurotus griseus*.[2]

*P. griseus* grew with medium rapidity on a thiamine-peptone agar[1] and a modified Czapek-Dox agar to which corn steep solids had been added. It formed a pink pigment which diffused into the agar. After the fungus had grown for one to several weeks, discs 5.5 mm. in diameter were cut on a radius extending from the center of the colony into the agar adjacent to the colony. These discs were placed on a yeast-peptone agar seeded with *Staph. aureus* and the plates incubated overnight at 37 ± 2°C. Clear zones of inhibition were found around the discs indicating that some substance or substances had diffused from the agar discs into the surrounding seeded medium in sufficient concentration to inhibit the growth of the staphylococci in the agar. A disc cut from the agar at as great a distance as 20 mm. from the edge of the colony frequently produced a zone of inhibition. The zones, while small, were clear, indicating that bacteria resistant to the antibiotic substance or substances were absent. Disc tests, with strains of *Staph. aureus* resistant to other antibiotic substances, indicated that *P. griseus* produced an antibiotic substance which was different from penicillin and different from the active substances produced by several other Basidiomycetes. The antibiotic substance or substances from *P. griseus* did not inhibit the growth of *Escherichia coli*.

*P. griseus* was grown on several types of nutrient media, differing from one another in the source of nitrogen and carbon, to determine the effect of the medium on the production of the antibacterial substances by the fungus. Cane sugar, dark brown sugar, galactose, lactose, mannitol and corn steep solids were ineffective carbon sources; maltose and soluble starch were poor; dextrose and levulose were the best and nearly equal so far as the

production of antibacterial substances was concerned. Various amounts of dextrose ranging from 10 to 100 g. per l. were tested; 40 g. per l. seemed somewhat superior to 20 or 60 g. Nitrates and asparagine in the presence of dextrose, were unsatisfactory sources of nitrogen; corn steep solids, an extract of corn steep solids made with 90 per cent methanol, N-Z-CASE, amigen and neopeptone were all effective. On the basis of this survey it was decided that a practical medium to use for liquid culture was a modified Czapek-Dox mineral solution[3] to which 40 g. of dextrose and 5 g. of corn steep solids were added per liter of solution.

*Antibiotic Material in Liquid Culture.*—The fungus was grown at 25°C. in Fernbach flasks on coils of beech shavings, using one liter of the above medium per flask. After a growth period of about one month, the fungous mat had nearly covered the entire liquid surface; the liquid had an activity of about 1000 dilution units per ml. when assayed with *Staph. aureus* (Heatley strain). The culture fluid was then decanted and replaced by one liter of fresh corn steep medium. After a further incubation period of from 5 to 7 days, the antibacterial activity of the culture fluid in the reflooded flask was usually at least 256 dilution units. This culture fluid was then decanted and replaced by fresh nutrient solution. This reflood technique saved from seven to fifteen days in the production of each lot of active culture fluid and was especially useful for those Basidiomycetes which grow slowly.[4] Flasks which had been reflooded nine times have shown as rapid and as great a production of antibacterial substance during the last period of reflooding as during the first.

*Isolation of an Antibiotic Substance.*—Pooled culture fluids with an activity of from 256 to 1024 dilution units per ml. were strained through cheesecloth to remove bits of mycelium and wood. They were then extracted by shaking with a one-tenth volume of chloroform. The chloroform-in-water emulsion was removed and centrifuged to break the emulsion. The chloroform portion was extracted three times with one-tenth volume of one per cent sodium bicarbonate solution to remove acids. The chloroform solution was then reduced to a few milliliters by distillation under reduced pressure, transferred to a small beaker and left at room temperature until all the chloroform had evaporated. A reddish gum in the beaker was dissolved in the minimum volume of hot ethanol, ether was added, the beaker covered and let stand at room temperature to permit slow evaporation of the solvents. Orange-colored crystals formed soon after the ether was added. The crystals were removed, washed with ether and air-dried. The product was recrystallized from a chloroform-ether solution. Large amber colored crystals were formed when evaporation of the solvents took place very slowly. Fine needle-like yellow crystals were formed as a result of rapid evaporation of the solvents. From 100 to 180 mg. of the crystalline material were obtained per l. of culture fluid.

A solution prepared from the crystals showed activity after 24 hrs. against *Staph. aureus* at a concentration of 1 μg. per ml. of solution and no activity against *E. coli*. The crystalline antibiotic substance was named pleurotin.

*Chemical Properties of Pleurotin.*—Pleurotin began to melt with decomposition at temperatures between 200° and 215°C. depending upon the rate of heating. Qualitative tests indicated the absence of ash and of the following elements: halogens, nitrogen and sulfur.

Microanalysis[9] gave the following: C, 70.83, H, 6.43; Mol. wt. (Rast) 343; MeO, 0.0. The computed values for an empirical formula of $C_{20}H_{22}O_5$ were C, 70.16; H, 6.47; Mol. wt. 342.4. Pleurotin was optically active with $\alpha]_D^{23°} = -20°$; C = 0.59 m. chloroform. We are indebted to Dr. J. D. Dutcher of The Squibb Institute for Medical Research for the molecular weight, analysis and optical rotation.

The absorption spectrum had a single absorption peak in the ultra violet at 2500 A for which the molecular extinction co-efficient was 13,680 (molecular weight assumed to be 342) for a solution in 4 per cent ethanol. The absorption in the visible was not measured.

The solubility of pleurotin at 25° was in water 0.125 mg. per ml., in 95% ethanol 6.8 mg. per ml., in 5% ethanol, 0.37 mg. per ml., in ether 3.5 mg. per ml. and in chloroform more than 200 mg. per ml. It was relatively insoluble in dilute acids, dilute solutions of sodium bicarbonate and in petroleum ether. It was more soluble in acetone.

Pleurotin did not give a color reaction with ferric chloride. It liberated iodine from acidified potassium iodide solution. Its reaction with a solution of potassium cyanide to give a blue color was used as the basis of a colorimetric method suitable for the quantitative determination of pleurotin in culture fluids and other solutions.

Pleurotin was adsorbed from culture fluids by Norit A and eluted from the air-dry carbon by chloroform. Pleurotin is a neutral substance which reacts with alkali to give an acidic product devoid of antibacterial activity.

Pleurotin was not thermostable. Solutions of pleurotin in 0.1 M. phosphate buffer when boiled for ten minutes lost 50 per cent of their biological activity at pH 3, 75 per cent at pH 6.5 and all their activity at pH 8.5 and higher. Pleurotin was 75 per cent destroyed in one hour at pH 8.5 and 25°C. An aqueous solution containing 100 μg. of pleurotin per ml. lost 30 per cent of its pleurotin as determined chemically after autoclaving at 120°C. for 15 min. Pleurotin in solution was rendered inactive by exposure to light for a few hours. It was filterable through a Seitz filter pad.

The chemical and physical properties of pleurotin are sufficient to establish it as different from all other antibiotic substances that have been prepared in pure form. The low solubility of pleurotin in aqueous solution and its instability suggest that its possible therapeutic value is unlikely.

*Antibacterial Action of Pleurotin.*—The antibacterial activity of pleurotin was determined by the methods in use in this laboratory.[6] The bacteria used were: *Staph. aureus, Klebsiella pneumoniae, Photobacterium fischeri,* and the following standard tester strains of S. A. Waksman: *E. coli, B. mycoides* and *B. subtilis.* The following table gives the minimum antibacterial concentrations of pleurotin after an incubation period of 24 hours:

| BACTERIA | μG. PER ML. |
|---|---|
| *Staphylococcus aureus* | 0.8 |
| *Bacillus mycoides* | 1.6 |
| *Bacillus subtilis* | 0.2 |
| *Escherichia coli* | 500.0 |
| *Klebsiella pneumoniae* | 500.0 |
| *Photobacterium fisheri* | 6.0 |

The activity for incubation periods of 16 to 18 hrs. was somewhat higher and for 48 hrs. was one-half or one-quarter the values given.

Pleurotin was active only on the gram-positive bacteria. The culture fluid from *Pleurotus griseus* was active only on the gram-positive bacteria indicating the absence of an appreciable amount of a second antibiotic substance with antibacterial properties markedly different from those of pleurotin. The antibacterial activity of a culture fluid was equal to that of a solution of pure pleurotin of the same concentration, the pleurotin content of the culture fluid being determined by the potassium cyanide method.

*Toxicity for Mice.*—Tests made by Dr. G. Rake at the Squibb Institute for Medical Research indicated that a single dose of pleurotin was not toxic when given intravenously to white mice at the rate of 24 mg. per kilogram of body weight. Pleurotin is so insoluble in aqueous solutions that larger amounts could not be given. For the twenty studies 11.4 mg. of pleurotin were dissolved in 1.0 ml. of warm ethanol and diluted with 19.0 ml. of warm saline solution. By maintaining the solution at 37° crystallization was delayed long enough to permit intravenous injection.

*Activity on M. tuberculosis.*—Through the courtesy of Dr. Ralph R. Mellon, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa., tests were carried out by Miss Jean Onslow on *Mycobacterium tuberculosis.* Discs cut from a 29-day old colony of *P. griseus* grown on the modified Czapek-Dox medium containing corn steep solids showed inhibition of an avirulent strain of *M. tuberculosis* H 234 on a yeast peptone agar. The activity was confined to the discs from the colony and was less than for *Staph. aereus.* A comparison for the two organisms is given in the following table where the inhibition in mm. is given for 6 discs taken on a radius extending from the center of the colony. Discs 1 and 2 came from the fungous colony.

| ORGANISM \ DISC | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| *Staph. aureus* | 17 | 17 | 11 | 9 | 8 | Halo |
| *M. tuberculosis H 234* | 11 | 7 | 0 | 0 | 0 | 0 |

Agar discs saturated with pleurotin produced no zones of inhibition. Pleurotin at a concentration of 100 μg. per ml. of Youmans' medium[7] or Kirchner's medium[8] was ineffective.

Discs from a colony *P. griseus* 9 days old were tested on virulent *M. tuberculosis* H 37 grown on Herrold egg medium. A disc taken from within the limits of the fungous colony produced an inhibition zone of 7 mm. Discs from the edge of the colony or in the agar adjacent to the colony were ineffective.

*Relative Activity of Pleurotus griseus and pleurotin.*—Some observations made during the course of our investigation indicated that all of the antibacterial activity of *P. griseus* was not accounted for by pleurotin. Disc cut from within the limits of a colony of *P. griseus* gave zones of inhibition on *Staph. aureus* and *M. tuberculosis* considerably larger than those obtained with similar discs saturated with pleurotin. The presence in the mycelium of an enzyme capable of forming an antibacterial substance during the incubation of the discs at 37° or of a factor which enhanced the activity of pleurotin was not demonstrated by our experiments.

On the other hand, an aqueous extract of the mycelium tested by the cup method against *Staph. aureus* was considerably more effective than a saturated solution of pleurotin and an acid fraction.[9] prepared from culture liquid gave large zones of inhibition.

The acid fraction showed some activity (1333 dilution units per g.) on *M. tuberculosis* H 234, although a saturated solution of pleurotin was inactive. The activity of this fraction on *Staph. aureus* was 8000 dilution units per g.

This evidence was taken to indicate the production by *P. griseus* of an antibacterial substance or substances other than pleurotin. Since the unidentified substances were not isolated, we do not know whether they fall within the antibiotic range (effective at a dilution of 1 to 40,000 or less). In any event, the quantity in the culture liquid must be small since within the limits of error the antibacterial activity of the culture liquid can be accounted for on the basis of its pleurotin content as determined chemically.

* This investigation was supported in part by grants from The Commonwealth Fund and The Squibb Institute for Medical Research.

[1] Robbins, W. J., Hervey, A., Davidson, R. W., Ma, R., and Robbins, W. C., *Bull. Torrey Bot. Club*, **72**, 165–190 (1945).

[2] The fungus was obtained from Dr. Ross W. Davidson and is numbered 14616-R in his collection. Another isolation of *Pleurotus griseus* obtained from Dr. Mildred K. Nobles was also active. No difference in the pleurotin formed by the two strains was detected.

[3] The mineral solution contained per liter, 3 g. $NaNO_3$, 1 g. $KH_2PO_4$, 0.5 g. KCl, 0.5 g. $MgSO_4.7H_2O$ and 0.01 g. $Fe_2(SO_4)_3$.

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[9] The acids removed from the chloroform extract of the culture liquid by treatment with $NaHCO_3$, as described previously, were dissolved in ether. The ether solution was extracted with 1 per cent $NaHCO_3$, the bicarbonate solution acidified and the organic acids passed into ether. This process was repeated nine times, a procedure which should have removed all pleurotin. The acid fraction obtained in this way was taken to dryness and extracted with water. The resulting water extract was the acid fraction referred to in the text.

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## *ANTIBIOTICS FROM BASIDIOMYCETES II. Polyporus biformis**

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In a survey of fungi reported earlier,[1] *Polyporus biformis*[2] was found to produce antibacterial substances. Its activity on *Staphylococcus aureus* (H) as evidenced by the disc test, encouraged us to investigate it further.

This fungus grew well at 25°C. on malt agar, thiamine-peptone agar and a modified Czapek-Dox agar to which corn steep solids had been added,[1] and produced antibacterial substances on all three media. Tested by the streak method, inhibition zones extending 12 to 25 mm. from the edge of the fungus colony were observed. The inhibition of *E. coli* was less than that of *Staph. aureus*. When the antibacterial activity was tested by the agar disc[1] method, inhibition areas as large as 25 mm. in diameter with a 5.5 mm. disc were obtained with *Staph. aureus*.

Culture liquids of *P. biformis* evidenced antibacterial activity. The fungus was grown at 25°C. in 2800 ml. Fernbach flasks containing 1 l. of modified Czapek-Dox medium with dextrose and corn steep solids[3] on coils of beech wood shavings which furnished mechanical support for the mycelium. After a growth period of 2 weeks or more, the activity of the culture liquid on *Staph. aureus* ranged from 64 to 256 dilution units. When the activity of the culture fluid approached 256 dilution units, the liquid was decanted with suitable precautions to prevent contamination of the cultures and each mycelial mat was reflooded with 1 l. of fresh sterile culture solution. Within 6 to 10 days the activity justified further decantation and reflooding. Several mats were reflooded as many as 20 times in the

course of a year with as rapid and as great production of antibacterial substances during the last reflood period as during the first. Maximum activity of culture liquid in reflooded flasks was reached in 2 weeks or less; extending the period to 8 weeks did not result in a material increase in activity. An extract of the mycelium prepared by grinding with sand and water exhibited considerable antibacterial activity for *Staph. aureus*.

Preliminary experiments showed that the antibacterial activity was increased 4 to 8 times by boiling the culture liquid for 10 minutes. For example, culture liquid with an activity for *Staph. aureus* of 64 dilution units per ml. was mixed with an equal volume of 0.1 M. phosphate buffers to produce pH 3.0 or pH 6.5. An equal volume of sodium bicarbonate-sodium carbonate buffer solution of pH 8.5 was added to some of the liquid and to another aliquot an equal volume of 2 per cent $Na_2CO_3$ to reach approximately pH 11.0. Each of these solutions was allowed to stand 1 hr. at 25°C., 1 hr. at 60°C. and 10 minutes at 100°C., neutralized and assayed. Boiling at 100°C. at pH 3.0 and 6.5 increased the activity on *Staph. aureus* 4 times, did not affect it at pH 8.5 and reduced it at pH 11.0. The following table in which the activity on *Staph. aureus* is given in dilution units per ml. summarizes the effects of pH and temperature on the culture liquid in this experiment.

| pH | 1 HR., 25°C. | 1 HR., 60°C. | 10 MINUTES, 100°C. |
|---|---|---|---|
| 3.0 | 32 | 64 | 128 |
| 6.5 | 64 | 32 | 256 |
| 8.5 | 16 | 32 | 16 |
| 11.0 | 16 | 2 | < 2 |

There appeared to be two antibiotic substances in the boiled culture liquid of *P. biformis;* one neutral in character; the other, acid. It was found that most of the active material could be extracted by organic solvents from slightly acid, neutral or slightly alkaline solutions; that is, it behaved as a neutral substance. However, by extracting the culture liquid twice with $CHCl_3$ at pH 6 to 7, acidifying the residual culture liquid to pH 2, or lower, and reëxtracting with $CHCl_3$, the presence of a small amount of an antibiotic substance acidic in nature was demonstrated. The neutral substance was named biformin and the acidic, biforminic acid.

It was found, further, that the antibacterial activity was completely lost if the culture liquid, or extracts from it, were dried. The $CHCl_3$ extracts, even though dried in a high vacuum, formed a black, insoluble product and lost their activity completely.

*Concentration of Antibacterial Substances.*—The concentration procedure eventually developed and generally used in our experiments was as follows: The pooled culture liquid (pH 4.9) from 20 Fernbach flasks was boiled for 10 minutes, cooled and extracted with 0.1 volume of $CHCl_3$ to remove biformin. The residual culture liquid was acidified to pH 2.0 or

less and reëxtracted with 0.1 volume of $CHCl_3$. This $CHCl_3$ solution contained the biforminic acid. The aqueous extracted solution had little activity and was discarded.

The $CHCl_3$ solution containing the biformin (neutral antibacterial substance) was evaporated at room temperature to small volume in a large crystallizing dish by blowing a stream of warm air from a hair-drier over the surface of the liquid. Before complete drying, the $CHCl_3$ was covered by a layer of water and evaporation continued until all of the chloroform was removed. The turbid, brownish aqueous solution was filtered to remove water-insoluble substances. The filtrate was clarified and sterilized by filtration through a Seitz sterilizing pad. The resulting pale yellow solution had an activity against *Staph. aureus* of from 900 to 1750 dilution units per mg. of solids.

The biformin was further purified by precipitating with silver nitrate and recovering it from the silver precipitate. Two procedures were followed, both of which appeared satisfactory. An aqueous solution of crude biformin, prepared as described above, was acidified with $HNO_3$ and silver nitrate was added. An alternate method was to stir a $CHCl_3$ extract of culture fluid containing biformin with one quarter volume of 0.17 per cent $AgNO_3$ for four hours.

The yellow silver compound was found to darken on drying and biformin could not be recovered from the brownish material. It seemed to be stable if kept under water. Biformin was recovered from the silver compound by suspending it in a large volume of water, stirring vigorously with an electric stirrer and adding KI and HCl slowly. Stirring was continued for an hour after the addition of the KI and HCl. The mixture of silver iodide, biformin and water was neutralized and extracted several times with ether. The ether was removed by rapid evaporation over a layer of water with stirring. The aqueous solution was filtered through a Seitz filter to remove finely divided solids and to sterilize the solution. A solution of biformin prepared in this way assayed 3600 dilution units per milligram against *Staph. aureus*.

The chloroform solution containing biforminic acid (the acid antibacterial substance) was extracted twice with one-tenth volume of 1 per cent sodium bicarbonate solution. The combined bicarbonate solutions were extracted twice with an equal volume of ether, which was discarded. The bicarbonate solution was then acidified to less than pH 2 and the turbid aqueous solution extracted three times with an equal volume of ether. The ether extracts were combined and washed several times with water. The biforminic acid was extracted from the ether solution by a small amount of one per cent sodium bicarbonate solution. This antibiotic substance was nearly as sensitive as biformin to drying.

The antibacterial activities of biformin and biforminic acid were similar. This was the justification for naming the acid substance biforminic acid.

Biforminic acid accounted for less than 10 per cent of the antibacterial activity of the boiled culture liquid. The biforminic acid content of the culture liquid, as far as our observations indicated, was not materially affected by boiling. The increased activity on boiling the culture liquid was the result of the formation of biformin. The precursor of biformin was not extracted by $CHCl_3$ or methyl isobutyl ketone and was inactive on *Staph. aureus*.

*Chemical Properties of Biformin.*—Biformin was found to be soluble in water, very soluble in ether, chloroform, methyl-isobutyl-ketone and alcohol. It formed nearly colorless solutions in water. It was not volatile with steam. Its activity was unaffected by heating to boiling in 0.01 N acid or alkali. Biformin was quite stable in dilute aqueous solution, but unstable in concentrated aqueous solution. Its instability on drying and in concentrated solution and its ability to form a silver compound, all suggest that it is a highly unsaturated substance. The black, insoluble product which resulted from drying biformin, was interpreted to be a polymerization product.

Neither biformin nor biforminic acid gave a colored compound with potassium cyanide solution. They did not release iodine from cold hydriodic acid. A biforminic acid preparation of low purity gave a brown color with aqueous ferric chloride; biformin did not react.

*Antibacterial Activity of Biformin and Biforminic Acid.*—Both biformin and biforminic acid were active against a wide variety of bacteria. The activities were determined by serial dilution and are given in the following table:

| | INHIBITORY CONCENTRATION IN μG. PER ML. | | |
|---|---|---|---|
| ORGANISM | BIFORMIN 121-3 | BIFORMIN 114-417 | BIFORMINIC ACID 112-71 |
| *Bacillus mycoides* | 5 | 13 | 3.4 |
| *Bacillus subtilis* | 0.04 | 0.04 | 0.2 |
| *Escherichia coli* | 1.6 | 0.6 | 3.4 |
| *Klebsiella pneumoniae* | 1.6 | 0.6 | 6.8 |
| *Photobacterium fischeri* | 0.02 | ... | ... |
| *Pseudomonas aeruginosa* | 50 | 32 | 220 |
| *Mycobacterium phlei* | 0.6 | > 2.5 | 0.8 |
| *Mycobacterium smegmatis (smegma)* | 3 | 3.5 | 6.8 |
| *Staphylococcus aureus* | 0.3 | 0.8 | 0.7 |

Results for two of the purest preparations of biformin prepared through the silver compound are given. The biforminic acid was a concentrate that had good activity. The results are reported as the minimum concentration in μg. per ml. that caused inhibition of growth of the test bacteria for 24 hours. The *Pseudomonas aeruginosa* was obtained through the courtesy of Merck & Co., Inc.; the *Mycobacterium phlei* was obtained from Dr. G. Rake; the *M. smegmatis* (*smegma*) was a strain that had been carried in culture on

an egg medium at Smith College for many years and was furnished by Dr. Elinor V. Smith.

*Pseud. aeruginosa* was the most resistant organism tested, requiring 32 to 50 μg. per ml. for inhibition. *B. subtilis, E. coli, K. pneumoniae, M. phlei, M. smegmatis* (*smegma*), *Photo. fischeri* and *Staph. aureus* were inhibited by less than 5 μg. per ml. *B. mycoides* was somewhat more resistant.

We were not successful in developing strains of *Staph. aureus* resistant to biformin.

*Antifungal Activity.*—*P. biformis* and biformin exhibited considerable antifungal activity. This was suggested by the observation that contamination of cultures of *P. biformis* after decanting culture liquid and reflooding was extremely rare. Inhibition of various fungi growing in the vicinity of colonies of *P. biformis* was observed. No growth of *Trichophyton mentagrophytes* occurred in a nutrient agar containing 4 dilution units (*Staph. aureus*) of biformin per ml.[4]

*Mycobacterium Tuberculosis.*—Through the courtesy of Dr. Ralph R. Mellon, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa., preliminary tests were carried out by Miss Jean Onslow on the activity of *P. biformis* on *M. tuberculosis*. Agar discs, cut from a 6-day old colony grown on Czapek-Dox agar medium supplemented with corn steep solids, inhibited an avirulent strain (H 234) of *M. tuberculosis* on a yeast-peptone medium. Discs from a 4-day old culture of the fungus were found to give zones of inhibition on virulent *M. tuberculosis* H 37 grown on Herrold egg agar. A comparison of the activity on *Staph. aureus* and *M. tuberculosis* on the two media is given in the following table. The discs were 5.5 mm. in diameter and cut on a radius extending from the center of the colony. The first two discs came from within the limits of the fungous colony. The diameter of the zones of inhibition is given in mm.

| ORGANISM AND MEDIUM \ DISC | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| *Staph. aureus* | | | | | | |
| Yeast peptone | 22 | 22 | 22 | 12 | Halo | |
| *M. tuberculosis* (H 234) | | | | | | |
| Yeast peptone | 18 | 15 | 11 | 9 | 0 | |
| *Staph. aureus* | | | | | | |
| Herrold egg agar | 9 | 9 | 0 | 0 | 0 | 0 |
| *M. tuberculosis* (H 37) | | | | | | |
| Herrold egg agar | 16 | 14 | 14 | 0 | 0 | 0 |

Boiled culture liquid with an activity of 256 dilution units for *Staph. aureus* showed some activity in Kirchner's medium for both strains of *M. tuberculosis*.

Further experiments[6] with a biformin sample prepared through the silver compound indicated the activity *in vitro* to be as great or greater on *M. tuberculosis* as on *Staph. aureus*. This is shown in the following table, in which the minimum inhibitory concentrations are given in μg. per ml.

| SAMPLE NO. | COMPOUND | *M. phlei* | *M. smegmatis* | *M. tuberculosis* H 37 Rv | *Staph. aureus* (*H*) |
|---|---|---|---|---|---|
| 121-3 | Biformin | 1.4 | 1.8 | 0.56 | 0.97 |

*Staph. aureus* was grown in beef heart broth and the Mycobacteria in a modified Kirchner's medium.

*Effect of Blood on Activity of Biformin.*—The addition of 5 per cent calf serum to beef heart broth had little or no effect on the activity of biformin on *Staph. aureus*. The addition of 5 per cent defibrinated rabbit blood reduced the activity to 0.1 that in the both.

*Animal Toxicity.*—Given intravenously, 10.7 mg./kg. of sample 121-3 caused no deaths in 5 mice used for each test. However, 12.5 mg./kg. of sample 121-3 killed 3 of 10, 15.0 mg./kg. killed 6 of 10 mice and 18.8 mg./kg. killed 5 of 5. Given intraperitoneally to small numbers of animals, 9.4 mg./kg. of sample 121-3 killed 4 of 5; with 12.5 mg./kg. of sample 121-3, 2 of 5 were killed and 37.5 mg./kg. killed 5 out of 5. At the lower dosage the deaths were delayed. Administered subcutaneously or intraperitoneally, 7.15 mg./kg. per day over a period of 5 days caused no deaths of 5 treated mice, while 4.68 mg./kg. per day, administered intraperitoneally to younger mice for 12 days, resulted in 1 death in 10 mice.

One of us (F. K.) suffered from severe dermatitis involving swelling and watery blisters after contact with preparations of biformin.

*Chemotherapeutic Action.*—No chemotherapeutic benefits were observed in mice of preparation 121-3 against *Staph. aureus* (Smith) or *M. tuberculosis* H 37 infections in mice. The lack of activity *in vivo* was probably related to the action of blood in reducing the activity of biformin (see above).

Preparation 121-3 was given intraperitoneally and subcutaneously at 7.5 mg./kg. in tests with *Staph. aureus* (Smith). The experiment was repeated with 3.75 mg./kg. intraperitoneally with one group of mice and 7.5 mg./kg. subcutaneously with another. In tests with *M. tuberculosis* preparation 121-3 was given intraperitoneally at 9.38 mg./kg. daily for 3 days and reduced to 4.69 mg./kg. per day until death of the animals. The experiment was repeated using an intraperitoneal injection of 2.5 to 3.75 mg./kg. daily.

*Summary.*—*Polyporus biformis* produces two antibiotic substances in culture liquids. These have been named biformin and biforminic acid. Both substances inhibit a number of gram negative, gram positive and acid-fast bacteria. The best preparations of biformin inhibited *Staphylococcus aureus* in beef broth at less than 1 μg. per ml. and were equally active on *Mycobacterium tuberculosis*. Serum did not affect the activity *in vitro* but

whole blood reduced the activity materially. No chemotherapeutic action was observed against *Staph. aureus* or *M. tuberculosis* infections in mice.

* This investigation was supported in part by grants from The Commonwealth Fund and The Squibb Institute for Medical Research.

[1] Robbins, W. J., Hervey, A., Davidson, R. W., Ma, R., and Robbins, W. C., *Bull. Torrey Bot. Club*, **72**, 165–190 (1945).

[2] This fungus was obtained from Ross W. Davidson and is numbered 71423R in his collection.

[3] Robbins, W. J., Kavanagh, F., and Hervey, A., these PROCEEDINGS, **33**, 171–176 (1947).

[4] The authors are indebted to Dr. Ilda McVeigh for this experiment.

[5] The authors are indebted to Miss C. M. McKee, The Division of Microbiology, The Squibb Institute for Medical Research, for assistance in these experiments and those on blood, animal toxicity and therapeutic action.

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## *THE POLE CELLS OF DIPTERA, THEIR FATE AND SIGNIFICANCE*

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Numerous investigations of the pole cells of Diptera, *Miastor, Chironomus, Calliphora, Lucilia* and *Drosophila* have amply verified the classical observations of Metschnikoff (1855, 1866), Leuckhart (1865) and Balbiani (1885) that these are the source of the germ cells in the gonads of flies.[1] The generalization of the early separation of germ and somatic lines of cells has become so firmly established among biologists that certain very fundamental facts concerning the pole cells have been overlooked in spite of a number of important pieces of information reported in recent investigations by Rabinowitz (1941), Sonnenblick (1941), and Aboim (1945).[2] The last named alone has recognized the possibility that some of the pole cells may have some other developmental fate than that classically demonstrated.

In the course of a detailed study of the normal embryology of *Drosophila* the author has obtained embryological and cytological evidence which establishes the pole cells in a new light and at the same time fills out our hitherto incomplete and unsatisfactory understanding of the nature and origin of the mid-gut of higher Diptera.

The formation of the pole cells in *Drosophila melanogaster* was first described by Huettner (1923). Subsequently Rabinowitz (1941) studied them in great detail providing data to show that far more pole cells are formed than ever enter the gonads. The number at the incipient blastoderm stage is 39–73, average 55. but prior to the invaginations which are the

most conspicuous feature of gastrulation, some of the pole cells migrate between the posterior blastoderm cells to the outer surface of the yolk mass, leaving 20–47, average 39, pole cells to be carried into the proctodaeal-amniotic invagination. Of these pole cells only slightly more than half are included in the gonads according to Sonnenblick (1941) and Aboim (1945). The non-included pole cells are not easily followed in iron hematoxylin preparations, and Sonnenblick suggests they are lost in the gut contained yolk. Rabinowitz states that the early migrating pole cells become lost and degenerate in the yolk. Aboim observed no signs of degenerating pole cells in the gut, but he was not able otherwise to establish their fate.

The observations here reported elucidate the fate of these "lost" pole cells and clarify the nature of the first pole cell movements between the blastoderm cells to the interior of the egg. A detailed account illustrated with photomicrographs will appear elsewhere. This report is based on a study of a series of sections of timed embryos of the Oregon R. wild stock of *D. melanogaster* which were silver impregnated by the Bodian technique, as described by Poulson (1945).[3] This method provides remarkably clear differentiation of various cell and tissue types. The pole cells stand out distinctively in such preparations and there is little likelihood of confusion with other cells, both with regard to nuclear structure and appearance of cytoplasm.

After the dorsal and lateral movement (7–9 hours) of the germinal pole cells into the mesoderm at the level of the 10th segment where they become incorporated in the gonads, the remaining pole cells are found in association with the posterior mid-gut rudiment. They come to take up a position at the anterior edge of the posterior rudiment as it moves to unite with the anterior rudiment of mid-gut between the 9th and 10th hours. They form two groups (on either side and not so noticeable in iron-hematoxylin preparations, but conspicuous in Bodian treated sections) at the points of union of the mid-gut rudiments. At first the groups look like small gonads, but this appearance rapidly changes as the mid-gut cells undergo the movements and form changes which complete the enclosure of the yolk. The pole cells retain their characteristic appearance, and it can be seen that at the time at which the sac-like gut begins to change its form to a long tube one of the first constrictions is at the level of the intestinal pole cells. With the completion of the form-change in the mid-gut the pole cells are seen to form the inner epithelium of the mid-section of the mid-gut. They remain characteristically different in size and form from the cells of the more anterior and posterior sections of the mid-gut and clearly correspond to the large cells of that region of the larval mid-gut designated by Marie Strasburger (1932) as the "Mitte."[4] Strasburger's observations on the physiology of this section of the gut set it off from the other parts of the mid-gut as the region of acid secretion. The physiological studies of Hobson (1931) on the

same region of the gut of *Lucilia* characterize it as a region of low pH (3.2) and of little or no proteolytic enzyme activity.[5] Studies of Waterhouse (1945) on the differential uptake of metallic ions by certain cells in this region of the mid-gut of *Lucilia cuprina* are of interest in view of its origin.[6] The fate of this region in metamorphosis to the adult requires careful study, although from Robertson's (1936) account of metamorphosis of the mid-gut[7] it seems likely that these cells are lost with most of the other larval mid-gut epithelial cells.

The early entry of pole cells between the posterior blastoderm cells to the interior is interpreted as the first of the series of movements in gastrulation in the eggs of *Drosophila* and other higher Diptera. These cells remain at the posterior surface of the yolk spreading out on it and later moving dorso-anteriorly with the extending embryo. They become incorporated also with the posterior rudiment of mid-gut and thus a part of the definitive larval intestine. They come to lie along with the "lost" pole cells in the middle region of the mid-gut and are difficult to distinguish from them.

Experimental proof of this should be obtainable in embryos and larvae in which the pole cells have been removed or killed by cautery or ultra-violet light; in these the middle section of the mid-gut must be reduced or missing. Such material is already in the hands of Geigy (1931) and Aboim (1945) who had followed the development of agametic gonads of *Drosophila* following ultra-violet treatment of pole cells.[8] It is hoped that this experimental evidence will soon be forthcoming.

It is no longer possible to interpret the posterior polar plasm, or oosome, and its included granules as germ cell determinants in the original sense of Hegner and his predecessors, or even in the modified sense of Huettner (1923). The pole cells must be regarded in a new light as potential, but not as completely determined, germ cells. The latter is true only when, as Aboim (1945) has so beautifully demonstrated, they enter the lateral mesoderm. When they do not, they become a specialized section of the mid-gut whose function may well repay careful investigation. Although no cytological differences have been demonstrated between those cells which are observed to leave the mid-gut and those which remain there, physiological and biochemical differences may well be sought at an early stage.

[1] For references and discussion of early literature on the pole cells see: Huettner, A. F., *Jour. Morph.*, **37**, 385–423 (1923); Wilson, E. B., *The Cell in Development and Heredity*, 3rd ed., Macmillan Co., New York, 1232 pp. (1928).

[2] Rabinowitz, M., *Jour. Morph.*, **69**, 1–49 (1941); Sonnenblick, B. P., these PROCEEDINGS, **27**, 484–489 (1941); Aboim, A. N., *Revue Suisse de Zool.*, **52**, 53–154 (1945).

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[4] Strasburger, Marie, *Z. f. wiss. Zoöl.*, **140**, 539–649 (1932).

[5] Hobson, R. P., *Jour. Exp. Biol.*, **8**, 109–123 (1931).

[6] Waterhouse, D. F., *Coun. Sci. Ind. Res.* (*Australia*), Bull. **191**, 1–20 (1945).

[7] Robertson, C. W., *Jour. Morph.*, **59**, 351–399 (1936).

[8] Geigy, R., *Revue Suisse de Zool.*, **38**, 187–288 (1931); Aboim, A. N., *loc. cit.*

# A GENERAL CLASS OF PROBLEMS IN CONFORMAL MAPPING

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A function $f(z)$ which is defined in the unit circle is said to be schlicht there if the relations $f(z_1) = f(z_2)$, $|z_1| < 1$, $|z_2| < 1$ imply that $z_1 = z_2$. We shall say that a function

$$f(z) = \sum_{\nu=1}^{\infty} a_\nu z^\nu, \qquad |z| < 1, \tag{1}$$

belongs to class $S$ if $f(z)$ is regular and schlicht in $|z| < 1$ and $a_1 = 1$.

Let $\mathbf{R}$ be a closed set lying in $|z| < 1$, and let $\Psi_\nu(\tau)$ be a measure function defined in the space $\mathbf{R}$. Given an integer $n$ there is a number $M = M_n$ such that $|f^{(\nu)}(z)| \leqq M$ for all $z \subset \mathbf{R}$ and for $\nu = 0, 1, 2, \ldots, n$ when $f(z)$ belongs to class $S$. Let $F_\nu(\zeta_0, \bar{\zeta}_0, \ldots, \zeta_n, \bar{\zeta}_n)$ denote a complex-valued function which is continuous together with its first-order partial derivatives in an open set containing the closed set $|\zeta_\nu| \leqq M$, $(\nu = 0, 1, \ldots, n)$.

Given the functions $F_1, F_2, \ldots F_m$ and the measure functions $\Psi_1, \Psi_2, \ldots, \Psi_m$, let

$$P_\nu = \int_{\mathbf{R}} F_\nu(f(z), f'(z), \ldots, f^{(n)}(z), \bar{f}^{(n)}(z)) d\Psi_\nu \tag{2}$$

for $\nu = 1, 2, 3, \ldots, m$. If $f(z)$ belongs to class $S$ then the point

$$P_f = (P_1, P_2, \ldots, P_m)$$

is a point in a Euclidean space of $2m$ real dimensions, and the point $P_f$ is said to belong to $f(z)$. As $f(z)$ ranges over $S$, the point $P_f$ belonging to $f(z)$ ranges over a set which we call $P$. Since the class $S$ is compact, the set $P$ is closed and bounded. The problem is to find the region $P$.

Many of the problems on schlicht functions which concern the values taken in the interior of the unit circle, as opposed to boundary-value problems, are subsumed under this general formulation. Since special problems included in this general statement can often be carried further toward complete solution, this note will discuss several specific problems of this type and it will be observed that the method applied to these problems can be extended, at least in part, to the general problem. The region of variability of the coefficients $(a_2, a_3, \ldots, a_n)$ of functions belonging to class $S$ is one problem of this type, and it has been discussed in a previous note by the authors.[1]

If $z_1$ is a point of $|z| < 1$, an important problem is to find the region of variability of $f'(z_1)$ when $f(z)$ belongs to class $S$. Let $f'(z_1) = p + iq$, where $p$ and $q$ are real. Then for fixed $z_1$, the point $(p, q)$ ranges over a

closed plane set $P$ when $f$ ranges over $S$. Let $G(p, q)$ be continuous together with its first order partial derivatives in an open set containing $P$, and let the gradient of $G$ be non-vanishing in $P$. Let the maximum of $G(p, q)$ in $P$ occur for a particular function $f(z) \subset S$.

In a paper to appear shortly, the authors show that if $(\alpha, \beta)$ is an analytic Jordan arc in $|z| < 1$ not passing through $z = 0$ or $z = z_1$, then for every sufficiently small complex number $\epsilon$ there is a function $f_\epsilon(z)$ which tends to $f(z)$ as $\epsilon$ tends to zero, and such that

$$f_\epsilon(z) = f(z) + \frac{\epsilon}{2\pi i}\int_\alpha^\beta \frac{p(u)}{2u^2}\left\{\left(\frac{uf'(u)}{f(u)}\right)^2 \frac{2f^2(z)}{f(u) - f(z)} - zf'(z)\frac{u+z}{u-z} + f(z)\right\} du + \frac{\bar{\epsilon}}{2\pi i}\int_\alpha^\beta \frac{\bar{p}(u)}{2\bar{u}^2}\left\{ zf'(z)\frac{1+\bar{u}z}{1-\bar{u}z} - f(z)\right\} d\bar{u} + o(\epsilon). \quad (3)$$

Here $p(u)$ is an arbitrary function which is analytic in a domain containing $(\alpha, \beta)$ and vanishes at $\alpha$ and $\beta$.

If $\Delta p + i\Delta q$ is the variation of $f'(z_1)$, then the variation of $G$ when $f(z)$ is replaced by $f_\epsilon(z)$ is given by

$$\Delta G = \frac{\partial G}{\partial p}\Delta p + \frac{\partial G}{\partial q}\Delta q + o(\epsilon)$$

or

$$\Delta G = Re\{G_1\ \Delta(f'(z_1))\} + o(\epsilon), \quad (4)$$

where

$$G_1 = \frac{\partial G}{\partial p} - i\frac{\partial G}{\partial q}.$$

The variation $\Delta(f'(z_1))$ of $f'(z_1)$ can be obtained by differentiating (3) with respect to $z$ and setting $z = z_1$. If we substitute this in relation (4) we find that $\Delta G$ is equal to the real part of an expression of the form

$$\frac{\epsilon}{2\pi i}\int_\alpha^\beta A(z_1, u)du + \frac{\epsilon}{2\pi i}\int_\alpha^\beta B(z_1, \bar{u})d\bar{u} + o(\epsilon).$$

We may replace the second term by its conjugate without changing the real part, so $\Delta G$ is, within an error $o(\epsilon)$, equal to the real part of

$$\frac{\epsilon}{2\pi i}\int_\alpha^\beta \frac{p(u)}{2u^2}\left\{\left(\frac{uf'(u)}{f(u)}\right)^2 P\frac{2f(u) - f(z_1)}{[f(u) - f(z_1)]^2} - Q\frac{u+z_1}{u-z_1} - \bar{Q}\frac{1+u\bar{z}_1}{1-u\bar{z}_1} - M\frac{2u - z_1}{(u-z_1)^2} - \bar{M}\frac{u(2-u\bar{z}_1)}{(1-u\bar{z}_1)^2}\right\} du. \quad (5)$$

Here $P = 2G_1f'(z_1)f(z_1)$, $Q = G_1z_1f''(z_1)$ and $M = 2G_1z_1f'(z_1)$. Since $G$ attains its maximum for the function $f(z)$, the real part of (5) is non-

negative for all small $\epsilon$ so (5) must be equal to zero. Since $p(u)$ is an arbitrary analytic function subject to the conditions stated, we see that $f(z)$ must satisfy the differential equation

$$P\left(\frac{zf'(z)}{f(z)}\right)^2 \frac{2f(z) - f(z_1)}{[f(z) - f'(z_1)]^2} = Q\frac{z + z_1}{z - z_1} + \bar{Q}\frac{1 + z\bar{z}_1}{1 - z\bar{z}_1} + M\frac{2z - z_1}{(z - z_1)^2} + \bar{M}\frac{z(2 - z\bar{z}_1)}{(1 - z\bar{z}_1)^2} \quad (6)$$

where $z$ has been written in place of $u$.

The right-hand side of (6) is real on the unit circle $|z| = 1$, and can be shown to be non-negative there with at least one zero of even order on $|z| = 1$. If we set $w = f(z)$, then this differential equation can be written in the form

$$A\left(z\frac{dw}{dz}\right)^2 \frac{2w - \alpha}{w^2(w - \alpha)^2} = \frac{(z - e^{i\psi})^2(z - z_2)(z\bar{z}_2 - 1)}{(z - z_1)^2(1 - z\bar{z}_1)^2}. \quad (7)$$

Here $A$, $\alpha$, $\psi$, $z_2$ are constants with $\psi$ real, $|z_2| \leqq 1$, and $\alpha = w(z_1)$. The conditions that $w(z)$ is normalized at the origin and that $(w - \alpha)/(z - z_1)$ approaches $w'(z_1)$ as $z$ approaches $z_1$ imply that

$$A = -\alpha e^{2i\omega} z_2 z_1^{-2}$$

and

$$A = \alpha(z_1 - z_2)(z_1\bar{z}_2 - 1)(z_1 - e^{i\Psi})^2 z_1^{-2}(1 - |z_1|^2)^{-2}.$$

The point $z_1$ can be taken real and positive without loss of generality.

The variables in equation (7) may be separated and the integrals expressed in terms of elementary functions. Thus the region of variability of $f'(z_1)$ is obtained exactly at all points of the unit circle $|z| < 1$. A function belonging to a boundary point of this region maps $|z| < 1$ onto the $w$-plane minus analytic slits which have at most a finite number of critical points. The authors hope to discuss this region in greater detail in a later paper.

If $f(z)$ belongs to class $S$ the area of the map by $w = f(z)$ of the circle $|z| \leqq a$, $0 < a < 1$, is given by the expression

$$A = \int_0^a \int_{-\pi}^{\pi} |f'(re^{i\theta})|^2 r\,dr\,d\theta = \frac{a}{4}\frac{d}{da}\int_{\pi}^{\pi} |f(ae^{i\theta})|^2 d\theta.$$

To illustrate the principle with equations which are not too complicated, consider the allied problem of maximizing the integral

$$B = \int_{-\pi}^{\pi} |f(ae^{i\theta})|^2 d\theta \quad (8)$$

when $f(z)$ ranges over $S$. Let the maximum of $B$ occur for a particular function $f(z) \subset S$. If the variation (3) is written in the form

$$f_\epsilon(z) = f(z) + \epsilon\Phi(z) + \bar{\epsilon}\Psi(z) + o(\epsilon)$$

then, substituting $f_\epsilon(z)$ for $f(z)$ in (8), one obtains

$$B_\epsilon = B + 2Re\{\epsilon \int_{-\pi}^{\pi} (\bar{f}\Phi + f\bar{\Psi})d\theta\} + o(\epsilon).$$

It therefore follows that

$$\int_{-\pi}^{\pi} (\bar{f}\Phi + f\bar{\Psi}\, d\theta) = 0$$

and if the function $p(u)$ is "peaked," it follows after some manipulation that

$$\left(\frac{zf'(z)}{f(z)}\right)^2 \int_{-\pi}^{\pi} \frac{2f(ae^{i\theta})\,|f(ae^{i\theta})|^2}{f(z) - f(ae^{i\theta})}\,d\theta = \int_{-\pi}^{\pi} \{-2|f(ae^{i\theta})|^2 + ae^{i\theta}\bar{f}'(ae^{i\theta})\bar{f}(ae^{i\theta})\frac{z + ae^{i\theta}}{z - ae^{i\theta}} + ae^{-i\theta}\bar{f}'(ae^{i\theta})f(ae^{i\theta})\frac{1 + zae^{-i\theta}}{1 - zae^{-i\theta}}\Big\}d\theta.$$

Here the independent variable $z$ has been written in place of $u$.

Several sub-classes of the class $S$ are often studied, of which we mention three:

$S_M$, the class of functions $f(z) \subset S$ which satisfy $|f(z)| \leqq M$ in $|z| < 1$, where $M$ is some constant exceeding 1.

$S_r$, the class of functions $f(z) \subset S$ which are real for real $z$.

$S_0$, the class of functions $f(z) \subset S$ which satisfy $f(-z) = -f(z)$.

For functions belonging to $S_M$ we have in place of (3)

$$f_\epsilon(z) = f(z) + \frac{\bar{\epsilon}}{2\pi i}\int_\alpha^\beta \frac{p(u)}{2u^2}\left\{\left(\frac{uf'(u)}{f(u)}\right)^2 \frac{2f^2(z)}{f(u) - f(z)} - zf'(z)\frac{u + z}{u - z} + f(z)\right\}du - \frac{\bar{\epsilon}}{2\pi i}\int_\alpha^\beta \frac{\bar{p}(u)}{2\bar{u}^2}\left\{\left(\frac{\bar{u}\bar{f}'(u)}{\bar{f}(u)}\right)^2 \frac{2\bar{f}(u)f(z)}{M^2 - \bar{f}(u)f(z)} - zf'(z)\frac{1 + \bar{u}z}{1 - \bar{u}z} + f(z)\right\}d\bar{u} + o(\epsilon), \quad (9)$$

where, however,

$$Re\left\{\frac{\epsilon}{2\pi i}\int_\alpha^\beta \frac{p(u)}{2u^2}\left[1 - \left(\frac{uf'(u)}{f(u)}\right)^2\right]du\right\} + o(\epsilon) \leqq 0. \quad (10)$$

Condition (10) restricts the small complex number $\epsilon$ to lie in a half-plane. Using (9) and (10), the differential equation satisfied by a function $(z)f$

of class $S_M$ which belongs to a boundary point of the region of variability of $f'(z_1)$, $f \subset S_M$, is of the form

$$\left(\frac{zf'(z)}{f(z)}\right)^2 \left\{\lambda \frac{2f(z) - \alpha}{(f(z) - \alpha)^2} + \bar{\lambda} \frac{f(z)(2M^2 - \bar{\alpha} f(z)}{(M^2 - \bar{\alpha} f(z))^2} + \rho\right\} =$$

$$\beta \frac{2z - z_1}{(z - z_1)^2} + \bar{\beta} \frac{2z - z^2\bar{z}_1}{(1 - z\bar{z}_1)^2} + \gamma \frac{z + z_1}{z - z_1} + \bar{\gamma} \frac{1 + z\bar{z}_1}{1 - z\bar{z}_1} + \rho$$

where $\alpha = f(z_1)$, $\lambda$, $\beta$ and $\gamma$ are complex constants and $\rho$ is a non-negative constant. The variation can also be modified such that, if $f(z)$ belongs to $S_r$ or to $S_0$, so does $f_\epsilon(z)$. Corresponding differential equations are then obtained for functions belonging to these classes.

The general problem stated in the beginning of this note leads to a differential equation for extremal schlicht functions which is of the form

$$\left(\frac{dw}{dz}\right)^2 N(w) = Q(z).$$

Here $N(w)$ and $Q(z)$ will be rational functions whenever (2) reduces to the form

$$P_\nu = \sum_{j=1}^{k} F_\nu(f(z_j), f'(z_j), \ldots, f^{(n)}(z_j), \bar{f}^{(n)}(z_j)) \qquad (13)$$

for $\nu = 1, 2, \ldots, m$. This is the most important case at the present time, and problems concerning the region of variability $P$ of the point $(P_1, P_2, \ldots, P_m)$, where $P_\nu$ is given by (13), can be further classified according to the order $n$ of the highest derivative involved.

* This note was written during the time that the authors were under contract with the Office of Naval Research.

[1] *The coefficients of schlicht functions* III, these PROCEEDINGS, **32**, 111–116 (1946).

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## *CONTINUOUS PARTICLES*

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*1. The Gravitational Field of an Uncharged Mass.*—In the theory of the electromagnetic field an antisymmetric tensor $F_{\mu\nu}$ is defined by the relations

$$F_{\mu\nu} = \frac{\partial \phi_\mu}{\partial x_\nu} - \frac{\partial \phi_\nu}{\partial x_\mu}, \qquad (1)$$

where $\phi_\mu$ is the vector potential. The charge and current vector is

$$J^\mu = F^{\mu\nu}{}_{,\nu}, \tag{2}$$

and the energy tensor is

$$U_\mu{}^\nu = -F^{\mu\alpha}F_{\nu\alpha} + {}^1/_4 g_\mu{}^\nu F^{\alpha\beta}F_{\alpha\beta}, \tag{3}$$

which gives

$$U_\mu{}^\nu{}_{,\nu} = F_{\mu\alpha}F^{\alpha\nu}{}_{,\nu} = F_{\mu\alpha}J^\alpha, \tag{4}$$

and also by contraction yields

$$U = 0. \tag{5}$$

We postulate the validity of the expression (3) for the gravitational energy tensor, subject to the supplementary condition

$$U_\mu{}^\nu{}_{,\nu} = 0 \tag{6}$$

corresponding to the fact that there is no electric charge. We postulate further that the existence of an electromagnetic field corresponds to the condition

$$U_\mu{}^\nu{}_{,\nu} \neq 0 \text{ at that point.} \tag{7}$$

Identifying therefore $U_\mu{}^\nu$ with the gravitational energy tensor $(-R_\mu{}^\nu + {}^1/_2 g_\mu{}^\nu R)$, we see that we must have $R = 0$, and hence $-R_\mu{}^\nu = U_\mu{}^\nu$, which shows that now $R_\mu{}^\nu{}_{,\nu} = 0$, in agreement with the Bianchi identities. The form (1) suggests the possibility of a torsional stress in addition to the radial stress. Denoting by $P$ and $\tau$ the radial and torsional stresses, respectively, we have the following scheme for $F_{\mu\nu}$:

$F_{\mu\nu} =$ ($\mu$ down, $\nu$ right)

| | | | |
|---|---|---|---|
| 0 | $\tau_3$ | $-\tau_2$ | $P_1$ |
| $-\tau_3$ | 0 | $\tau_1$ | $P_2$ |
| $\tau_2$ | $-\tau_1$ | 0 | $P_3$ |
| $-P_1$ | $-P_2$ | $-P_3$ | 0 |

(8)

Consider the case where there is no torsional stress. We can write the interval around a particle with spherical symmetry in the form:

$$ds^2 = -e^\lambda dr^2 - r^2 d\theta^2 - r^2 \sin^2\theta d\varphi^2 + e^\nu dt^2. \tag{9}$$

The only components of $F_{\mu\nu}$ being $F_{14} = -F_{41} = P_1$, we get from (3),

$$-R_1{}^1 = R_2{}^2 = R_3{}^3 = -R_4{}^4 = {}^1/_2 P_1{}^2 e^{-\lambda-\nu}. \tag{10}$$

The equality $R_1{}^1 = R_4{}^4$ leads to the relation $\lambda' = -\nu'$; substituting this

result in the differential equation $R = 0$, we obtain, replacing $e^{\nu}$ by $\gamma$, the expression[1]

$$\gamma = 1 - \frac{2m}{r} + \frac{A}{r^2},$$

where $m$ is the mass of the particle and $A$ is a constant to be determined. We shall determine $A$ by the condition that the integral of the energy density over the whole of space shall be equal to $m$, with a numerical factor depending on the units chosen. We have $R^{44} = -\frac{A}{r^4\gamma}$ and

$$-\int R^{44}\sqrt{-g}dV = 4\pi A\int_0^{\infty}\frac{dr}{r^2 - 2mr + A} =$$

$$4\pi A\frac{1}{\sqrt{A - m^2}}\left(\frac{\pi}{2} + \text{arc tan}\frac{m}{\sqrt{A - m^2}}\right). \quad (11)$$

Setting $A = 2m^2$, this expression becomes equal to $6\pi^2 m$, and we obtain thus for $\gamma$,

$$\gamma = 1 - \frac{2m}{r} + \frac{2m^2}{r^2}. \quad (12)$$

The particle appears, therefore, as a continuous distribution of energy through space, the energy density being finite at every point, and tending to zero at infinity, the greater part of the mass being concentrated near the origin. We remark that for $r < 2m$ the field, given by $\frac{1}{2}\gamma'$, becomes repulsive. For $r < m$, there is a compression, and for $r > m$, there is a dilatation. The numerical value of $r$ for which $\gamma = 1$, is, for a neutron equal to

$$1.23 \times 10^{-52}\text{ cm}. \quad (13)$$

The expression for $\phi_4$, is determined by the condition $F^{4\nu}{}_{,\nu} = 0$ which is equivalent to

$$\frac{\partial}{\partial r}\left(\sqrt{-g}\frac{\partial\phi_4}{\partial r}\right) = 0$$

or

$$\frac{\partial}{\partial r}\left(r^2\frac{\partial\phi_4}{\partial r}\right) = 0,$$

which gives

$$\phi_4 = \frac{2m}{r}. \quad (14)$$

Calculating the value of the invariant $R_{\mu\nu}R^{\mu\nu}$ we find that it is equal to $(2m/r^2)^4$, which shows that $\phi = 2m/r$ is an invariant function of position.

If we take into account all the components of the vector potential $\phi_\nu$, we are led to consider the identification of the torsional stress with the spin. The value of $-R_4{}^4$ being $\frac{2m^2}{r^4}$, if the energy of the torsional stress can also be put in the form $\frac{s}{r^4}$, then the integral of the energy $\int \frac{s}{r^4}\sqrt{-g}dV$ would take the form $\frac{s}{m}$, so that the torsional energy would be inversely proportional to the mass of the particle, as is the case for the spin.

*2. The Electron.*—If we consider the mass of the electron to be wholly due to its electric energy, we must determine $F^{\mu\nu}$ by the two conditions

$$\int J^4\sqrt{-g}dV = e \tag{15}$$

and

$$\int {}^1/_2E^2\sqrt{-g}dV = m, \tag{16}$$

where $E$ is the electrostatic field of the electron, and $J^4$ the charge density. We have $E = F_{14} = -F_{41}$; also $J^4 = F^{4\nu}{}_{,\nu}$, and the $g_{\mu\nu}$ are Galilean. The two conditions are satisfied by the potential

$$\phi_4 = \frac{1}{2k}\log\eta = \frac{1}{2k}\log\left(1 - \frac{2ke}{r} + \frac{2k^2e^2}{r^2}\right), \tag{17}$$

where $k$ is a constant equal to $3.8 \times 10^{-4}$. The field is given by $\frac{1}{2k}\frac{\eta'}{\eta}$ and reduces to the Coulomb field at large distances. The charge and the energy densities are seen to be finite at every point, and tend to zero at infinity. The electron appears thus to be a continuous distribution of charge through space.

We remark that for a positive charge the field changes sign at a distance $r = 3.7 \times 10^{-13}$ cm., and that two positive charges should therefore attract each other in the range $3.7 \times 10^{-13}$ cm. to $1.8 \times 10^{-13}$ cm.

The electrostatic stress at a point may be considered as a transverse stress on a narrow tube situated on the radius vector.

Moreover if the radial stress of an uncharged mass has a transverse Poisson effect, then a neutron might be expected to have an attraction for a positive charge.

*3. Waves in Particles.*—The wave equation of Maxwell's theory is

$$g^{\alpha,\beta}(\phi_\mu)_{\alpha\beta} = J_\mu - R_\mu{}^\alpha\phi_\alpha. \tag{18}$$

For an uncharged particle $J_\mu = 0$. Moreover, in regions of weak fields $R_\mu{}^\alpha$ may be neglected, and the wave equation reduces to

$$\Box\,\phi_\mu = 0 \tag{19}$$

A solution of this equation is

$$\phi_\mu = \int_0^\infty dk \sum_{l=0}^{\infty} a_{klm} P_l^m \cos\theta e^{im\varphi} r^{-1/2} [\alpha J_{l+1/2}(kr) + \beta J_{-l-1/2}(kr)] e^{ikct} \tag{20}$$

Imagine an observer $0_1$ whose position coincides at time $t$ with the center 0 of a particle in relative motion with respect to the observer. The form of $\phi$ will not depend on the angle $\varphi$, but it may depend on the angle $\theta$. Let us assume that it does not depend on $\theta$. In that case we have $l = 0$, and the solution becomes:

$$\phi = \int_0^\infty a_k r^{-1/2} [\alpha J_{1/2}(kr) + \beta J_{-1/2}(kr)] e^{ikct} dk. \tag{21}$$

If we want to express this in exponential form we set $\alpha = i$, $\beta = -1$ so that

$$\phi = \int_0^\infty a_k \frac{e^{ik(r+ct)}}{kr} dk \tag{22}$$

Let us consider first $(a)$ as a constant not depending on $k$. The solution will then give us again the potential $\frac{1}{r}$, already obtained for the static case. We must therefore assume that $a_k$ will be found by the observer $0_1$ to be a function of $k$. We assume for $a_k$ the form

$$a_k = \frac{1}{1 - \frac{k}{k_1}} \tag{23}$$

where $k_1$ depends on the momentum of the particle and the nature of the medium. We obtain thus

$$\phi = \frac{A}{r} \int_0^\infty \frac{e^{ik(r+ct)}}{k - k_1} dk. \tag{24}$$

A contour integration[2] around the point $k_1$ gives

$$\phi = \frac{A}{r} \left[ -2\pi \sin k_1(r + ct) + \int_0^\infty \frac{\cos k(r + ct)}{k + k_1} dk \right] \tag{25}$$

the last integral diminishes very rapidly at distances of the order of $\frac{\pi}{k_1}$ so that at a distance of half a wave-length from the center of a moving particle a decreasing harmonic wave form is fully established and we have simply

$$\phi = -\frac{2\pi A}{r} \sin k_1(r + ct). \tag{26}$$

The particle thus appears to be surrounded by a spherical wave pattern which escorts it in its motion. The common velocity of the infinite number of waves which constitute the wave pattern, including the principal wave $k_1$, is $c$.

Substituting the form (26) in the wave equation $\Box\phi = 0$ we obtain, if $k_1$ does not depend on time, the equation $\Delta\phi + k_1^2\phi = 0$. If now we set $\lambda_1 = \frac{h}{mv}$, in accordance with the results of experiment this expression becomes $\Delta\phi + \frac{4\pi^2m^2v^2}{h^2}\phi = 0$, or, replacing $\frac{1}{2}mv^2$ by $E - U$ in the case of small velocities,

$$\Delta\phi + \frac{8\pi^2m}{h^2}(E - U)\phi = 0 \qquad (27)$$

where $E$ is the total and $U$ the potential energy of the particle.

*Summary.*—A reinterpretation of Maxwell's equations in general relativity leads to the deduction of a metric tensor for the field of an uncharged particle which yields a finite density of energy at every point in space, the integral of the energy over the whole of space being equal to the mass of the particle. The particle thus appears to be a continuous distribution of energy through space.

An expression is deduced for the electrostatic energy of an electron giving charge and energy densities which are finite at every point in space, the integrals over the whole of space being, respectively, equal to the charge and to the mass of the electron. An indication is found of the possibility of an attraction between two positive charges in a certain range of distance, and of the possibility of an attraction between a positive charge and an uncharged mass.

The consideration of a certain solution of the wave equation of Maxwell's theory leads to the notion of a harmonic spherical wave surrounding a particle in motion. This leads to a new interpretation of Schrödinger's wave equation.

[1] This form was obtained by Nordstrom and by Jeffery, who interpreted the constant $A$ as an electric charge; see Eddington, *Math. Th. Rel.*, p. 185.

[2] Lamb, *Hydrodynamics*, p. 401.

# RADIO-WAVE PROPAGATION AND ELECTROMAGNETIC SURFACE WAVES

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*1. A Discrepancy and Its Resolution.*—The problem of the propagation of radio-waves along the surface of the (plane) earth was first treated in a celebrated paper by A. Sommerfeld (1909).[1] Let the $(r, \varphi)$-plane of a cylindric system of coördinates coincide with the plane of the earth surface and let the $z$-axis point vertically upward. Sommerfeld considered a vertically oscillating dipole (radio-antenna) in the air, close to the origin ($z = 0$, $r = 0$) and asked what secondary waves were produced by the discontinuity due to the presence of the partially conducting earth. His investigation led to the result that the Hertzian vector $\Pi$ describing the electromagnetic field can be divided, in either medium (air and earth), into two parts: $\Pi = Q + P$, where the part $Q$ has the character of *space-waves*, which at large distances from the origin are proportional to $R^{-1}$, if $R = (r^2 + z^2)^{1/2}$. On the other hand, $P$ is a *surface-wave:* at large distances it becomes proportional to $r^{-1/2}$ and is restricted to the vicinity of the earth surface.

Ten years later (1919) the problem was re-examined by H. Weyl[2] who used a somewhat different mathematical approach. He obtained a solution which was identical with Sommerfeld's space waves $Q$ but which did not contain the surface wave $P$.

The reason for this discrepancy has never been satisfactorily explained. Since Weyl's method seemed mathematically simpler his result was favored by public opinion in numerous papers by other authors. Finally, in 1935, Sommerfeld himself conceded that the surface wave has no reality.[3] Referring to F. Noether,[4] he attributed this to an inaccuracy in the evaluation of his general solution. This evaluation consisted in carrying out a contour integration in which a pole of the integrand yielded the surface-wave $P$ and two branch-cuts accounted for the space-wave $Q$. According to Noether's explanation the pole is so close to one of the branch-cuts that the method of integration used by Sommerfeld, possibly, was not sufficiently reliable.

The question has not only historical interest but retains even now some actuality because Sommerfeld's method was recently applied by C. Y. Fu[5] to the analysis of the propagation of seismic waves. In this case, the singularities, instead of nearly coalescing, as in Sommerfeld's problem, are rather far apart so that the evaluation does not present any difficulties. If Noether's suggestion is the complete explanation for the absence of

surface waves in the electrodynamic disturbances, it should not apply to seismic waves. In other words, an oscillating elastic dipole within the earth would produce, among other things, seismic surface waves. It is, therefore, important to decide what the true nature of the discrepancy is and whether the above explanation is exhaustive.

We shall show in the next section that Noether's explanation is both insufficient and unnecessary. The difficulties already arise (and can be resolved) when only the general solution is considered, and before any evaluation is attempted. It seems that the resolution of the discrepancy has been delayed so long because of the mental attitude of all involved which led them to take it for granted that one of the conflicting solutions must contain a mathematical error. This is not so: from the mathematical point of view, both Sommerfeld's and Weyl's solutions are unimpeachable. However, they represent two different physical phenomena. On the one hand, Weyl's solution ($Q$) corresponds just to the wave of the oscillating dipole with its secondary space waves due to reflexion and transmission. On the other hand, Sommerfeld's solution ($Q + P$) is the superposition of two independent physical systems as follows: (1) the oscillating dipole with its secondaries ($Q$), (2) an electrodynamic surface wave ($P$). These two systems stand in no causal relation to each other, their yoking together in one mathematical expression is purely accidental. The fact that the space-waves $Q$, as evaluated by Sommerfeld, are identical with those found by Weyl does not bear out Noether's suggestion that the evaluation is at fault. On the contrary, Sommerfeld's evaluation seems to be entirely adequate.

*2. Mathematical Proof.*—Sommerfeld starts from the representation of the $z$-component of the Hertzian function for an oscillating dipole, $\Pi_0 = \exp.(ikR)/R$ in the form of the integral

$$\Pi_0 = \frac{1}{2}\int H(\lambda r)\exp.(\mp\sigma z)\sigma^{-1}\lambda d\lambda, \qquad (1)$$

$$\sigma = (\lambda^2 - k^2)^{1/2},\ \sigma' = (\lambda^2 - k'^2)^{1/2}.$$

We designate here by $k$ and $k'$ the wave-numbers of the upper and lower medium (air and earth), while $H$ denotes Hankel's cylindric function of the first kind and of order zero. The upper sign of the exponent refers to the case $z > 0$, the lower to $z < 0$, while the signs of the roots must be chosen so as to make the real parts of $\sigma$ and $\sigma'$ positive. The path of integration ($L$) in the $\lambda$-plane is the real axis from $-\infty$ to $+\infty$.

The Hertzian function determines the field components in the following way

$$\boldsymbol{E} = \boldsymbol{\Pi}k^2 + \nabla(\nabla\cdot\boldsymbol{\Pi}), \qquad \boldsymbol{H} = -ik^2c\omega^{-1}\nabla\times\boldsymbol{\Pi}, \qquad (2)$$

where $c$ is the velocity of light, $\omega$ the frequency, and the time factor exp.

$(-i\omega t)$ is omitted. This leads to the border conditions at the surface of the earth ($z = 0$),

$$k^2\Pi - k'^2\Pi' = 0, \quad \partial(\Pi - \Pi')/\partial z = 0, \tag{3}$$

where $\Pi$ is the total Hertzian function in the upper medium, $\Pi'$ in the lower.

To satisfy the border conditions, Sommerfeld assumes the existence of a *reflected* Hertzian function in the upper medium, $\Pi_R = \Pi - \Pi_0$, and of a *transmitted* one $\Pi'$, in the lower, which differ from the expression (1) mainly by the respective factors $f(\lambda)$ and $f'(\lambda)$ in the integrands. These expressions satisfy the wave equations $\nabla^2\Pi + k^2\Pi = 0$ and $\nabla^2\Pi' + k'^2\Pi' = 0$, while the factors can be chosen so as to fulfill the border conditions. Thus the total Hertzians in the two media become

$$\Pi = {}^1/_2 \int H(\lambda r) \exp.\ (-\sigma z)\sigma^{-1}[1 + f(\lambda)]\lambda d\lambda, \tag{4}$$

$$\Pi' = {}^1/_2 \int H(\lambda r) \exp.\ (\sigma' z)\sigma^{-1}f'(\lambda)\lambda d\lambda, \tag{5}$$

the paths of integration being the same as in $\Pi_0$. With the help of the border conditions the factors are found to be

$$1 + f(\lambda) = (k^2/k'^2)f'(\lambda) = (k^2\sigma' - k'^2\sigma)/(k^2\sigma' + k'^2\sigma). \tag{6}$$

The singularities of the integrands of (4) and (5) are represented in Fig. 1. They consist of the branch points, $\lambda = k$, $\lambda = k'$, and of the pole, $\lambda = k_0$, of the expression (6). The path of integration ($L$) can be displaced and is equivalent to two loops $Q_1$ and $Q_2$ around the branch cuts and to the residuum at the pole. As we mentioned in Section 1, the loops represent the space-waves $Q = (Q_1 + Q_2)$, the residuum the surface-wave $P$.

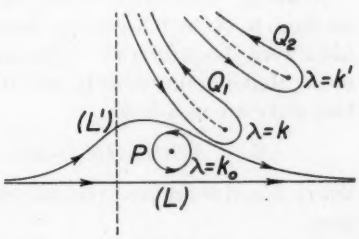


FIGURE 1.

It should be noticed, however, that the integrand of the expression (1) representing the original dipole does not have any singularity at the point $\lambda = k_0$. Hence, the path of integration of this integral can be displaced into the curve ($L'$) passing above the point $\lambda = k_0$. Since all that is required of the expressions (4) and (5) is that they satisfy the respective wave equations and the border-conditions, the integrals in them can be also conducted over the path ($L'$). Thus, in addition to the $L$-solution discussed above we have a second solution which we shall call the $L'$-solution. It is given by the integrals (4) and (5) conducted over the path $L'$. The evaluation of the $L'$-solution can be effected in the same

way, by displacing the path of integration upward. It is thus equivalent to the two loops about the branch cuts (which represent the space waves) and it does *not* include the residuum of the pole (i.e., it does not contain the surface-wave $P$).

The existence of the second solution was heretofore overlooked. Drawing attention to it is the essential contribution of this article. From a mathematical point of view, the existence of two different solutions is not at all surprising because it is well known that the integral of the equation $\nabla^2\Pi + k^2\Pi$, for given border conditions, is not unique.[6] Since the $L$-solution and the $L'$-solution each satisfy the differential equations and the border conditions of the problem, it follows that their difference must also satisfy the same conditions and represent a third possible solution. This is given by the integrals (4) and (5) conducted over the paths $+L$ and $-L'$, which are equivalent to a circuit about the pole $\lambda = k_0$ or to the residuum in this pole. We know already that this residuum represents the surface wave $P$ of Sommerfeld. We find, in this way, that the surface wave satisfies independently all the conditions of the problem and can exist for itself without any connection with the oscillating pole. This result is not entirely new since in another connection Sommerfeld himself had recognized the independent existence of *surface waves*.[7]

However, Sommerfeld considered there only plane waves while here we have to do with a circular surface-wave. Therefore, it will be well to say a word about this case. The simplest expressions for the $z$-components of the Hertzian functions $\Pi_s$ and $\Pi_s'$, (in the two media) of a circular surface-wave are as follows

$$\Pi_s = AH(\lambda r)\exp.(-\sigma z), \qquad \Pi_s' = BH(\lambda r)\exp.(\sigma' z), \tag{7}$$

where $A$ and $B$ are two constant coefficients; the other components being zero.

The border conditions (3) take then the form

$$k^2A - k'^2B = 0, \qquad \sigma A + \sigma' B = 0.$$

The two equations are compatible only when their determinant vanishes,

$$k'^2\sigma + k^2\sigma' = 0. \tag{8}$$

Hence, the value which the parameter $\lambda$ must be given in the expressions (7) is the root of the eq. (8). This root, $\lambda = k_0$, is identical with the pole of the functions (6). Therefore, it is easy to see that the expressions (7) become identical with the residua of the functions (4) and (5) for the pole $\lambda = k_0$, when the constants $A$, $B$ are suitably chosen. In other words, they are identical with Sommerfeld's surface wave $P$.

From the physical point of view, the most interesting of our results is the existence of the $L'$-solution. As this solution contains the oscillating

dipole but not the surface wave, it shows conclusively that the latter wave is not a part of the dipole radiation and is not generated by the dipole. This is true not only for electrodynamic but also for elastic oscillating dipoles which also do not generate (seismic) surface waves. Yet, apart from dipoles, the surface waves can exist and in seismology they are regularly observed in connection with earthquakes. The question how they are generated is an important one but it lies outside the scope of this article.

[1] Sommerfeld, A., *Annalen. Physik*, **28**, 665 (1909).

[2] Weyl, H., *Annalen. Physik*, **60**, 481 (1919).

[3] Sommerfeld, A., contribution to "*Frank-Mises, Differential- und Integral-gleichungen. Zweiter Teil*," p. 932 (Braunschweig, 1935).

[4] Noether, F., *Funktionentheorie und ihre Anwendungen in der Technik*, 165 (1931). The reference was not accessible to the author.

[5] Fu, C. Y., *Geophysics*, **12**, 57 (1947).

[6] Sommerfeld attempted a proof of the uniqueness (reference 1, pp. 680–682). However, this proof breaks down for wave functions which possess source singularities, as both the oscillating dipole and the surface wave do. In this respect the wave equation is in no way different from the Laplacean equation.

[7] Frank-Mises, *loc. cit.*, 877, 878, 930.